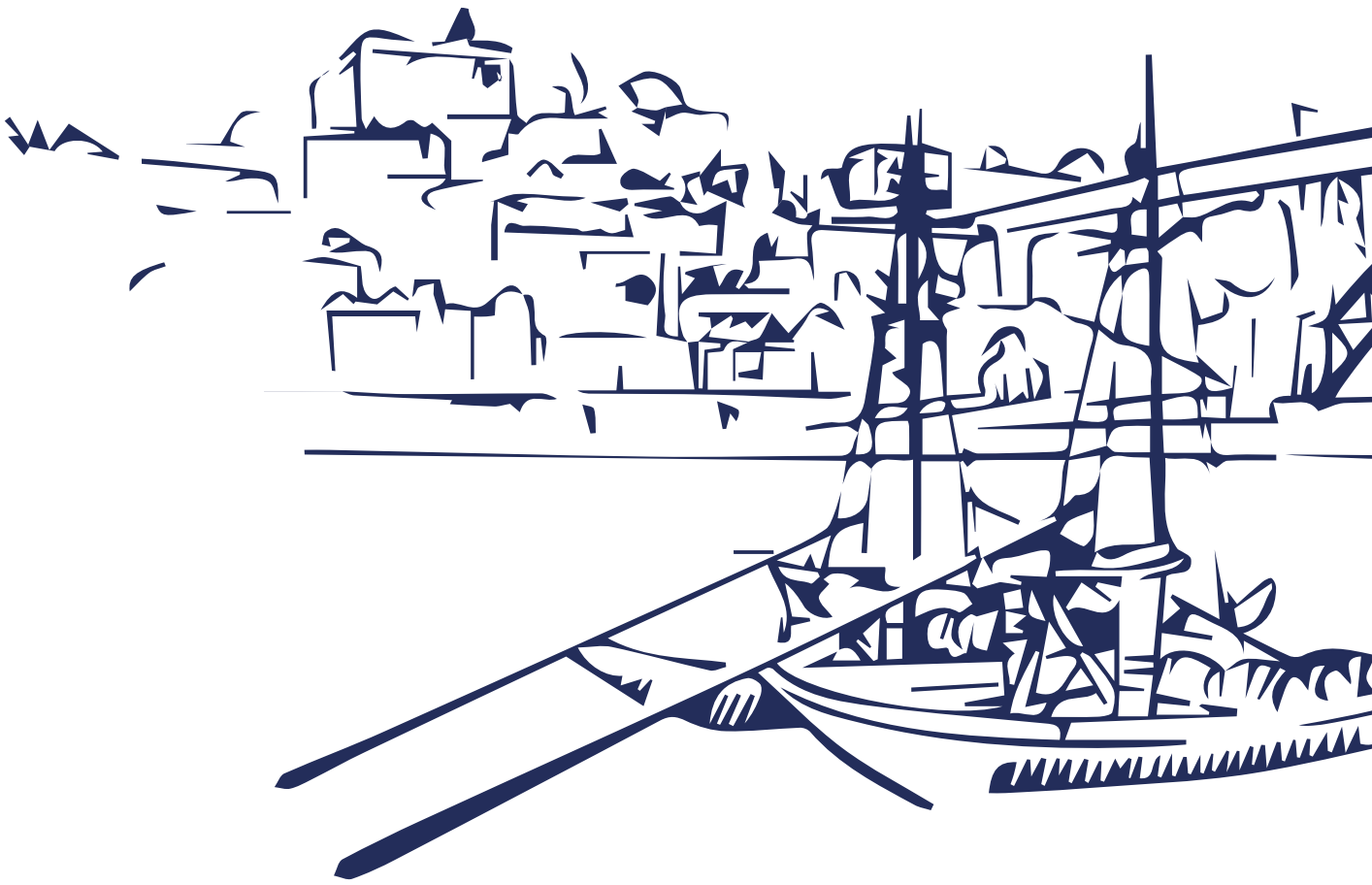
XVII CONGRESS OF THE IBERIAN SOCIETY OF CYTOMETRY



INSTITUTO PORTUGUÊS
DE ONCOLOGIA DO PORTO

14 - 18 JUNE 2021

VIRTUAL EDITION

CONTENTS

01. WELCOME MESSAGES

JULIA ALMEIDA PARRA
GABRIELA MARTINS

WELCOME MESSAGES

Dear colleagues, dear friends,

The COVID19 pandemic has an enormous global impact, including a high impact on the scientific community; consequently, to protect general health and wellbeing, both the SIC Board and the Local Organizing Committee agreed to organize the XVII Congress of the Iberian Society for Cytometry in a virtual format, which is really a challenge for all of us, but particularly for the organizers. Thus, we are very grateful for all the work done and to be done by the Organizing Committee of our SIC2021 Congress, chaired by Gabriela Martins.

SIC Meetings have been during many years a core event for networking and interaction for Spanish and Portuguese cytometrists, through promotion of education and scientific exchange in the fields of basic, translational and clinical applications in cytometry, and we hope these general goals are also achieved in a virtual environment. Hosting a virtual congress brings challenges but at the same time opens new possibilities; thus, although face-to-face interactions are an important aspect of any meeting, our goal is to offer a virtual meeting that is dynamic and (almost) as full as our in person event, through implementation of creative and novel digital tools.

I am sure that SIC2021 Meeting will bring people together even in a virtual environment, and I encourage you to have an active and enthusiastic participation. We are all committed to making this new format a resounding success.

With warm regards,

Julia Almeida Parra
President of Iberian Society for Cytometry

Dear colleagues and friends,

It is with great pleasure that we invite you, on behalf of the Board of the Iberian Society of Cytometry (SIC), the Organizing Committee and the Scientific Committee, to the XVII Congress of the Iberian Society of Cytometry, to be held from 14th to 18th June, 2021, in Porto (Portugal). We are greatly honoured to take on the responsibility given to us by the SIC Board. It has been quite a challenge to organize a virtual event, but we believe our new platform will allow us to have an interactive experience, as close as possible to a conventional congress.

We will keep our programme, including educational and plenary sessions, conferences and symposia. Digital tools will provide the same opportunities for discussion in real time. It will certainly be an intuitive and dynamic experience, whether on the computer or on the smartphone.

This virtual platform will also allow us to expand the scope of our scientific programme, with the participation of national and international expert speakers and research teams.

Porto is a beautiful city: a place of culture and knowledge that would be the perfect venue to host this Congress. Even though the evolving COVID-19 pandemic has limited us in many ways, our wish is to welcome you to Porto in the near future.

For now, it will be wonderful to meet you in this new reality of digital events. We would like to thank you for your presence in the XVII SIC Congress, and we truly hope you enjoy attending it.

Gabriela Martins
Chair Organizing Committee

02. **COMMITTEES**

ORGANIZING COMMITTEE
SCIENTIFIC COMMITTEE
ORGANIZATION

ORGANIZING COMMITTEE

Gabriela Martins
CHAIR

Artur Paiva
CO-CHAIR

Julia Almeida Parra
CO-CHAIR

Margarida Lima
CO-CHAIR

Ana Marta Pires

Carla Azevedo

Carlos Palmeira

Joana Caetano

João Pedro Barreto

José Carlos Segovia

Maria Emília Sousa

Maria Inês Godinho

SCIENTIFIC COMMITTEE

Alberto Órfão de Matos

Artur Paiva

Bruno Costa Silva

Carlos Fernández Giménez

Carlos Mendes

Carlos Palmeira

Carmen Jerónimo

Catarina Martins

Cidália Pina-Vaz

Gabriela Martins

Joana Caetano

Jordi Petriz

José Enrique O'Connor

José Mário Mariz

Julia Almeida Parra

Lúcio Lara Santos

Luís Araújo

Manuel Teixeira

Margarida Lima

Maria Jorge Arroz

Martín Perez Andrés

Paula Oliveira

Rui Gardner

Rui Medeiros

Vítor Vasconcelos

ORGANIZATION

Sociedad Ibérica de Citometría

IPOPORTO

Centro Hospitalar Universitário do Porto

CHUC
CENTRO HOSPITALAR E UNIVERSITÁRIO DE COIMBRA

03. **PROGRAMME**

SHORT VERSION

14TH JUNE - PRE CONGRESS COURSE

15TH JUNE - CONGRESS

16TH JUNE - CONGRESS

17TH JUNE - CONGRESS

18TH JUNE - CONGRESS

14TH JUNE

17:00H 20:30H

PRE-CONGRESS COURSE 1

FROM TECHNICAL ASPECTS TO AUTOMATED DATA ANALYSIS AND BEYOND

Coordinators: **Carlos Palmeira** (Porto, Portugal); **Alfonso Blanco** (Dublin, Ireland)

17:10h – 17:45h — FLOW CONCEPTS: HOW DOES A CELL BECOME A PLOT?
Alexandre Salvador (Porto, Portugal)

17:45h – 18:20h — MULTICOLOR PHENOTYPING: THE PATH TO DESIGN AND SUCCEED
Lola Martinez (Madrid, Spain)

18:20h – 18:30h — **Discussion/Questions**

18:30h – 18:45h — **Break**

18:45h – 19:20h — FLOW BEYOND PHENOTYPING: PROBING CELLULAR PROCESSES USING FLOW CYTOMETRY
Timothy Bushnell (New York, USA)

19:20h – 19:55h — LIGHT UP YOUR RESULTS! APPLY DATA ANALYSIS AND MANAGEMENT SMARTLY
Zaida Vergara (Madrid, Spain)

19:55h – 20:30h — **Discussion/Questions**

17:00H 20:45H

PRE-CONGRESS COURSE 2

LEUKAEMIA AND LYMPHOMA IMMUNOPHENOTYPING: THE BASICS

Coordinators: **Gabriela Martins** (Porto, Portugal); **Sérgio Chacim** (Porto, Portugal)

17:10h – 18:00h — EPIDEMIOLOGICAL ASPECTS FOR LYMPHOMA AND LEUKAEMIA, ITS CLINICAL PRESENTATION AND STRATEGIES FOR DIAGNOSIS
Sérgio Chacim (Porto, Portugal)

18:00h – 19:00h — ACUTE LEUKAEMIA: IMMUNOPHENOTYPIC ANALYSIS AND INTERPRETATION
Carlos Fernández Giménez (Salamanca, Spain)

19:00h – 19:15h — **Break**

19:15h – 20:15h — MATURE B CELL LYMPHOMAS: IMMUNOPHENOTYPIC ANALYSIS AND INTERPRETATION
Joana Caetano (Lisbon, Portugal)

20:15h – 20:45h — **Discussion/Questions**

17:00H 20:30H

PRE-CONGRESS COURSE 3

WHAT YOU SHOULD KNOW ABOUT IMMUNODEFICIENCIES: A CLINICAL AND CYTOMETRIC APPROACH

Coordinators: **Esmeralda Neves** (Porto, Portugal); **António Marinho** (Porto, Portugal)

17:10h – 17:45h — AN INTRODUCTION TO INBORN ERRORS OF IMMUNITY
Cátia Iracema Morais (Porto, Portugal)

17:45h – 18:20h — WHAT YOU SHOULD KNOW ABOUT IMMUNODEFICIENCIES: A CLINICAL AND CYTOMETRIC APPROACH
Martin Perez Andrés (Salamanca, Spain)

18:20h – 18:30h — **Discussion/Questions**

18:30h – 18:45h — **Break**

18:45h – 19:20h — EMERGENCIES ASSOCIATED TO INBORN ERRORS OF IMMUNITY: THE ROLE OF THE FLOW CYTOMETRY WITH PRACTICAL CASE ANALYSIS
Catarina Martins (Lisbon, Portugal)

19:20h – 19:55h — CLINICAL AND LABORATORY DIAGNOSTIC APPROACH OF COMMON VARIABLE IMMUNODEFICIENCY (CVID)
Kissy Annet Guevara-Hoyer (Madrid, Spain)

19:55h – 20:30h — **Discussion/Questions**

17:00H 20:30H

PRE-CONGRESS COURSE 4

NANOCYTOMETRY, CELL SORTING AND FUNCTIONAL ASSAYS

Coordinators: **Artur Paiva** (Coimbra, Portugal); **Bruno Costa Silva** (Lisbon, Portugal)

17:10h – 17:55h — INTRO TO SMALL PARTICLE DETECTION USING FLOW CYTOMETRY
Rui Gardner (New York, USA)

17:55h – 18:40h — HOW TO APPROACH SORTING OF BIONANOPARTICLES. VIRUSES AND EXTRACELLULAR VESICLES
Òscar Fornas (Barcelona, Spain)

18:40h – 18:55h — **Discussion/Questions**

18:55h – 19:10h — **Break**

19:10h – 19:55h — FUNCTIONAL ANALYSIS OF SUBCELLULAR COMPONENTS AND EXTRACELLULAR VESICLES BY CYTOMICS
José Enrique O'Connor (Valencia, Spain)

19:55h – 20:30h — **Discussion/Questions**

17:00H 20:45H

PRE-CONGRESS COURSE 5

AUTOMATIC ANALYSIS AT DIAGNOSIS AND FOLLOW-UP

Coordinators: **José Carlos Segovia** (Madrid, Spain); **Maria Jorge Arroz** (Lisbon, Portugal)

17:10h – 17:55h — THE USE OF REFERENCE DATABASES IN AUTOMATIC ANALYSIS: THEORETICAL BASES
Ana Bento (Salamanca, Spain)

17:55h – 18:05h — **Discussion/Questions**

18:05h – 18:20h — **Break**

18:20h – 19:20h — APPLICATIONS OF AUTOMATIC ANALYSIS IN THE SCREENING OF CHRONIC LYMPHOPROLIFERATIVE NEOPLASIA AND ACUTE LEUKAEMIA; ANALYSIS OF CLINICAL CASES
Paula Fernández (Aarau, Switzerland)

19:20h – 20:20h — APPLICATIONS OF AUTOMATIC ANALYSIS IN MRD TESTING IN B-ALL AND MULTIPLE MYELOMA; ANALYSIS OF CLINICAL CASES
Juan Flores Montero (Salamanca, Spain)

20:20h – 20:45h — **Discussion/Questions**

17:00H
20:45H

PRE-CONGRESS COURSE 6

FLOW CYTOMETRY IN ANIMAL EXPERIMENTAL RESEARCH

Coordinators: **Rita Ferreira** (Aveiro, Portugal); **Fátima Gärtner** (Porto, Portugal)

17:10h - 17:50h — THE USE OF ANIMAL MODELS IN MEDICAL RESEARCH: FROM THE LEGAL ASPECTS TO ITS PRACTICAL EXECUTION
Paula Oliveira (Vila Real, Portugal)

17:50h - 18:30h — USING AN INTEGRATED SOP APPROACH TO DESIGN FLOW CYTOMETRY EXPERIMENTS FOR ANIMAL RESEARCH
Jane Srivastava (California, USA)

18:30h - 18:45h — **Discussion/Questions**

18:45h - 19:00h — **Break**

19:00h - 19:40h — DISSECTING THE DIVERSITY OF MOUSE THYMIC CELLS BY FLOW CYTOMETRY
Pedro Ferreirinha (Porto, Portugal)

19:40h - 20:20h — PROFILING OF IMMUNE-ONCOLOGY AGENTS IN SYNGENEIC TUMOUR MODELS
Lukasz Magiera (Cambridge, UK)

20:20h - 20:45h — **Discussion/Questions**

15TH JUNE

WELCOME | ROOM 1

16:00H
16:25H

Gabriela Martins, Chair of Organizing Committee of the XVII Congress of Iberian Society
Julia Almeida, President of the Iberian Society of Flow Cytometry
Rui Henrique, Chairman of the Board of Directors of Porto Portuguese Oncology Institute
Margarida Lima, Co-chair of Organizing Committee of the XVII Congress of Iberian Society
Artur Paiva, Co-chair of Organizing Committee of the XVII Congress of Iberian Society

OPENING CONFERENCE | ROOM 1

16:25h - 17:10h

MONITORING OF THERAPY IN HEMATOLOGICAL MALIGNANCIES: CAN WE MOVE FROM BONE MARROW TO BLOOD?

Alberto Orfão (Salamanca, Spain)

17:10h - 17:20h — Panel Discussion

PARALLEL SESSION | ROOM 1

17:20H
18:40H

HEMATOLOGY 1: ADVANCES IN DIAGNOSIS (PART 1)

Chair: **Margarida Lima** (Porto, Portugal); **Rui Henrique** (Porto, Portugal)

17:30h - 18:00h — IMMUNOPHENOTYPIC IDENTIFICATION OF SÉZARY CELLS IN BLOOD USING EUROFLOW STRATEGIES
Julia Almeida (Salamanca, Spain)

18:00h - 18:20h — APPLICATIONS OF FLOW CYTOMETRY TO THE DIAGNOSIS OF HEREDITARY PLATELET DISORDERS
Catarina Lau (Porto, Portugal)

18:20h - 18:30h — **OP.01 //** ANTI-TRBC1 ANTIBODY-BASED DETECTION FOR ASSESSMENT OF T-CELL CLONALITY IN DIAGNOSTIC T-CELL FLOW CYTOMETRY PANELS: STANDARDIZATION OF SAMPLE PREPARATION AND ANALYSIS OF THE SPECIFICITY AND SENSITIVITY OF THE APPROACH
Noemi Muñoz Garcia (Salamanca, Spain)

18:30h - 18:40h — **Panel Discussion**

PARALLEL SESSION | ROOM 2

17:20H
19:10H

MICROBIOLOGY: CLINICAL AND FLOW CYTOMETRY APPLICATION

Chair: **José Enrique O'Connor** (Valencia, Spain); **Rui Medeiros** (Porto, Portugal)

17:30h - 18:00h — FLOW CYTOMETRY ON CLINICAL MICROBIOLOGY LAB. WHY NOT?
Cidália Pina Vaz (Porto, Portugal)

18:00h - 18:20h — EVALUATION OF AN ULTRA-RAPID FLOW CYTOMETRIC ANTIMICROBIAL ASSAY DIRECTLY FROM BLOOD CULTURES
Ana Dias (Porto, Portugal)

18:20h - 18:40h — COLISTIN ACTIVITY EVALUATION THROUGH FLOW CYTOMETRY
Daniela Fonseca e Silva (Porto, Portugal)

18:40h - 18:50h — **OP.02 //** AN ULTRA-RAPID FLOW CYTOMETRIC ASSAY FOR METHICILLIN RESISTANCE DETECTION AND VANCOMYCIN MIC DETERMINATION IN STAPHYLOCOCCUS AUREUS
Cidália Pina Vaz (Porto, Portugal)

18:50h - 19:10h — **Panel Discussion**

18:40h - 18:55h — **BREAK | ROOM 1**
(virtual commercial exhibition break)

PARALLEL SESSION | ROOM 1

18:55H
20:40H

HEMATOLOGY 1: ADVANCES IN DIAGNOSIS (PART 2)

Chair: **Paulo Lúcio** (Lisbon, Portugal), **Maria José Oliveira** (Porto, Portugal)

19:05h - 19:35h — ACUTE MYELOID LEUKEMIA WITH RECURRENT IMMUNOPHENOTYPIC ABNORMALITIES
Sergio Matarraz (Salamanca, Spain)

19:35h - 20:00h — PROBING THE BONE MARROW MICROENVIRONMENT IN LEUKEMIA
Delfim Duarte (Porto, Portugal)

20:00h - 20:10h — **Panel Discussion**

20:10h - 20:20h — **OP.03 //** MUTATIONAL PROFILE AND IMMUNOPHENOTYPIC CHANGES IN BONE MARROW COMPARTMENTS OF CHRONIC MYELOMONOCYTIC LEUKEMIA PATIENTS
João Gaião Santos (Coimbra, Portugal)

20:20h - 20:30h — **OP.04 //** MONITORING THE IMMUNOPHENOTYPE DURING THERAPY WITH 5-AZACITIDINE MAY ASSIST IN REDEFINING THE QUALITY OF RESPONSE IN PATIENTS WITH HIGH-RISK MDS
Dolores Subirá (Guadalajara, Spain)

20:30h - 20:40h — **Panel Discussion**

20:40h - 21:25h — **GECLID MEETING | ROOM 1**

16TH JUNE

14:30H 15:30H — ROOM 1

SIC WORKING GROUPS

14:30h - 15:00h — RECOMMENDATIONS FOR PNH SCREENING BY FLOW CYTOMETRY. RESULTS OF THE SIC PNH GROUP
Martin Perez Andrés (Salamanca, Spain)

15:00h - 15:15h — **Panel Discussion**

15:15h - 15:30h — UPDATE OF THE SIC WORKING GROUPS FOR HEMATOLOGICAL MALIGNANCIES
Martin Perez Andrés (Salamanca, Spain); **Alba Torres Valle** (Salamanca, Spain)

16:00H 17:05H — PLENARY SESSION | ROOM 1

SARS-COV2 AND FLOW CYTOMETRY

Chair: **Jordi Petriz** (Barcelona, Spain); **Andrea Cossarizza** (Modena, Italy)

16:10h - 16:55h — FLOW CYTOMETRY MONITORING OF SARS-COV-2 INFECTION REVEALS IMMUNE PROFILES AND KINETICS ASSOCIATED WITH DISEASE SEVERITY
Julia Almeida (Salamanca, Spain)

16:55h - 17:05h — **Panel Discussion**

17:05H 19:25H — PARALLEL SESSION | ROOM 1

IMMUNOLOGY 1: IMMUNE MONITORING

Chair: **Artur Paiva** (Coimbra, Portugal); **Maria José Oliveira** (Porto, Portugal)

17:15h - 17:45h — NEW STRATEGIES FOR B-CELLS MONITORING IN INFECTION, AUTOIMMUNITY AND IMMUNODEFICIENCIES
Martin Perez Andrés (Salamanca, Spain)

17:45h - 18:15h — SINGLE CELL APPROACHES FOR T CELL MONITORING IN VIRAL INFECTIONS AND CANCER
Sara De Biasi (Modela, Italy)

18:15h - 18:45h — IMMUNOPHENOTYPE OF BLOOD MONOCYTE AND DENDRITIC CELL SUBSETS
Daniela Damasceno (Salamanca, Spain)

18:45h - 18:55h — **Panel Discussion**

18:55h - 19:05h — **OP.05 //** IMMUNOLOGICAL PARAMETER RECOVERY AFTER ACTIVE COVID-19 DISEASE
David San Segundo (Santander, Spain)

19:05h - 19:15h — **OP.06 //** DEEP IMMUNE CELL PROFILING IN BLOOD OF MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE (MGUS)
Oihane Pérez Escurza (Salamanca, Spain)

19:15h - 19:25h — **Panel Discussion**

17:05H 19:20H — PARALLEL SESSION | ROOM 2

FLOW CYTOMETRY IN ANIMAL AND OCEANIC SCIENCES

Chair: **Vitor Vasconcelos** (Porto, Portugal); **Ana Faustino** (Évora, Portugal); **José Carlos Segovia** (Madrid, Spain)

17:15h - 17:40h — FLOW CYTOMETRY IN VETERINARY ONCOLOGY
Fulvio Riondato (Turin, Italy)

17:40h - 18:00h — ISOLATION AND IMMUNOPHENOTYPING OF PERIPHERAL BLOOD AND INTRAEPITHELIAL AND LAMINA PROPRIA
Beatriz Agulla Pérez (Madrid, Spain)

18:00h - 18:15h — **Panel Discussion**

18:15h - 18:35h — MARINE VIRAL ECOLOGY THROUGH THE BEAM OF FLOW CYTOMETRY
Gonçalo Piedade (Texel, Netherlands)

18:35h - 18:55h — HOW AUTOMATED FLOW CYTOMETRY CAN IMPROVE OUR UNDERSTANDING OF THE PLANCTON STRUCTURE, DISTRIBUTION AND FUNCTIONING
Gérald Grégori (Marseille, France)

18:55h - 19:05h — **Panel Discussion**

19:05h - 19:15h — **OP.07 //** DETECTION BY FLOW CYTOMETRY OF ALTERATIONS OF IMMUNE- AND PLATELET FUNCTION RELATED TO STRESS OR PATHOLOGIES IN MARINE MAMMALS
Mar Felipo Benavent (Valencia, Spain)

19:15h - 19:20h — **Panel Discussion**

19:20h - 19:35h — **BREAK** | ROOM 2
(virtual commercial exhibition break)

19:25h - 19:40h — **BREAK** | ROOM 1
(virtual commercial exhibition break)

19:40H 21:30H — PARALLEL SESSION | ROOM 1

HEMATOLOGY 2: MEASURABLE RESIDUAL DISEASE EVALUATION BY FLOW CYTOMETRY: ADVANCES AND NEW PERSPECTIVES

Chair: **António Campos** (Porto, Portugal); **Maria Jorge Arroz** (Lisbon, Portugal); **Fernanda Trigo** (Porto, Portugal)

19:50h - 20:20h — CURRENT FLOW CYTOMETRIC APPROACHES TO MEASURABLE RESIDUAL DISEASE DETECTION ON HEMATOLOGIC MALIGNANCIES
Juan Flores Montero (Salamanca, Spain)

20:20h - 20:50h — MRD DETECTION: WHAT IS NEW IN AML AND MM
Bruno Paiva (Pamplona, Spain)

20:50h - 21:00h — **Panel Discussion**

21:00h - 21:10h — **OP.08 //** BONE MARROW FOLLICULAR-LIKE T CELLS IN MONOCLONAL GAMMOPATHIES
Ana Silva (Coimbra, Portugal)

21:10h - 21:20h — **OP.09 //** ACUTE LEUKEMIA IN A 1 DAY-OLD NEWBORN
Ana Catarina Caldas Dias (Viseu, Portugal)

21:20h - 21:30h — **Panel Discussion**

19:35H 21:10H — PARALLEL SESSION | ROOM 2

FLOW CYTOMETRY IN BIOTECHNOLOGY

Chair: **Rui Gardner** (New York, USA), **Lillian Barros** (Bragança, Portugal)

19:45h - 20:10h — APPLICATION OF FLOW CYTOMETRY IN THE ASSESSMENT OF NATURAL PRODUCTS BIOACTIVITIES
Rui Abreu (Bragança, Portugal)

20:10h - 20:35h — FLOW CYTOMETRY IN FOOD MICROBIOLOGY AS RELATED TO CHEESE PRODUCTION
Martin Wilkinson (Limerick, Ireland)

20:35h - 20:55h — SURVIVAL AND METABOLISM OF HYDROXYCINNAMIC ACIDS BY DEKKERA BRUXELLENSIS IN MONOVARIETAL WINES
José António Couto (Porto, Portugal)

20:55h - 21:10h — **Panel Discussion**

17TH JUNE

16:00H 18:30H — PARALLEL SESSION | ROOM 1

FLOW CYTOMETRY IN SOLID TUMORS

Chair: **Júlio Oliveira** (Porto, Portugal); **Carmen Jerónimo** (Porto, Portugal)

16:10h - 16:35h — PREPARE TO EXPLORE THE TUMOR MICROENVIRONMENT
Martijn Van Baalen (Amsterdam, Netherlands)

16:00H 18:40H — PARALLEL SESSION | ROOM 2

NEW FRONTIERS IN FCM APPLICATIONS

Chair: **Alfonso Blanco** (Dublin, Ireland); **Paula Ludovico** (Braga, Portugal)

16:10h - 16:40h — NEW FRONTIERS IN FCM APPLICATIONS
Paul Robinson (Indiana, USA)

16:35h - 17:05h
THE POTENTIAL OF FLOW CYTOMETRY FOR IMMUNOSCORE ANALYSIS OF HUMAN LUNG TUMORS
Alexandre Corthay (Oslo, Norway)

17:05h - 17:35h
ANALYSES OF THE PERIPHERAL IMMUNOME IN CANCER IMMUNOTHERAPY TRIALS
Renee Donahue (Bethesda, USA)

17:35h - 17:50h
Panel Discussion

17:50h - 18:00h
IOP.01 // CHARACTERIZATION OF INTRATUMORAL INNATE LYMPHOCYTE POPULATIONS
Margareta Correia (Porto, Portugal)

18:00h - 18:10h
IOP.02 // UNRAVELLING THE BLADDER TUMOUR MICROENVIRONMENT USING THE FLOW CYTOMETRY TOOLBOX
Andreia Peixoto (Porto, Portugal)

18:10h - 18:20h
IOP.03 // THE APPLICATION OF FLOW CYTOMETRY IN TESTING NEW THERAPEUTIC STRATEGIES IN PRECLINICAL IN VIVO MODELS
Carlos Palmeira (Porto, Portugal)

18:20h - 18:30h
Panel Discussion

16:40h - 17:10h
FLOW KARYOTYPING AND CHROMOSOME SORTING
Òscar Fornas (Barcelona, Spain)

17:10h - 17:40h
FLOWFISH: ASSESSMENT OF RELATIVE TELOMERE LENGTH BY FLOW CYTOMETRY
Cláudia Nóbrega (Braga, Portugal)

17:40h - 18:10h
CYTOMICS IN IMMUNOTHERAPY TREATMENT DECISION-MAKING
Jordi Petriz (Barcelona, Spain)

18:10h - 18:25h
Panel Discussion

18:25h - 18:35h
OP.10 // EXPERIMENTAL AND DATA PROCESSING WORKFLOW FOR LARGE-SCALE IMMUNE MONITORING STUDIES BY MASS CYTOMETRY
Concepción Marañón (Granada, Spain)

18:35h - 18:45h
OP.11 // FLOWCT FOR DECONVOLUTION OF LARGE IMMUNOPHENOTYPIC DATASETS AND BIOMARKER DISCOVERY IN CANCER IMMUNOLOGY
Juan José Garcés (Pamplona, Spain)

18:45h - 18:50h
Panel Discussion

18:30h - 18:45h
BREAK | ROOM 1
(virtual commercial exhibition break)

18:50h - 19:05h
BREAK | ROOM 2
(virtual commercial exhibition break)

PARALLEL SESSION | ROOM 1

18:45H 20:40H

EXTRACELLULAR VESICLES, PROGRESSION AND TREATMENT RESISTANCE IN CANCER

Chair: **Lúcio Lara Santos** (Porto, Portugal); **Helena Vasconcelos** (Porto, Portugal)

18:55h - 19:25h
EMPLOYING FLOW CYTOMETRY TO EXTRACELLULAR VESICLES POPULATION ANALYSIS
Bruno Costa Silva (Lisbon, Portugal)

19:25h - 19:55h
UNDERSTANDING CELL–NANOPARTICLE INTERACTION: A NEW GATEWAY TO DISEASE THERAPEUTICS
Sofia Antunes Costa Lima (Porto, Portugal)

19:55h - 20:50h
Panel Discussion

20:10h - 20:20h
IOP.04 // THE POTENTIAL OF EXTRACELLULAR VESICLES CARGO AS MOLECULAR BIOMARKERS FOR CLEAR CELL RENAL CARCINOMA PATIENT'S MANAGEMENT
Francisca Dias (Porto, Portugal)

PARALLEL SESSION | ROOM 2

19:05H 21:05H

ACCREDITATION AND CERTIFICATION

Chair: **Juana Ciudad** (Salamanca, Spain); **Tiago Guimarães** (Porto, Portugal)

19:15h - 19:40h
KEY PERFORMANCE INDICATORS IN FLOW CYTOMETRY ASSAYS
Juana Ciudad (Salamanca, Spain)

19:40h - 20:05h
REVISITING RISK MANAGEMENT IN THE LIGHT OF THE COVID-19 PANDEMIC: STRATEGIES AND TOOLS TO IMPROVE SAFETY AND QUALITY IN FLOW CYTOMETRY LABORATORIES
Catarina Martins (Lisbon, Portugal)

20:05h - 20:30h
HOW TO ADAPT AND RISE TO A PANDEMIC
Lola Martinez (Madrid, Spain)

20:30h - 20:55h
BIOSAFETY GUIDELINES FOR THE MANAGEMENT OF POTENTIALLY BIOHAZARDOUS SAMPLES - SARS COV2
Óscar González (Salamanca, Spain)

20:55h - 21:05h
Panel Discussion

20:20h - 20:30h
OP.12 // URINE CYTOMETRY FOR NON-INVASIVE DIAGNOSTIC OF RENAL PATHOLOGY ASSOCIATED TO SYSTEMIC AUTOIMMUNE DISEASES
Francisco Pérez Cózar (Granada, Spain)

20:30h - 20:40h
Panel Discussion

20:40h - 21:25h
GENERAL ASSEMBLY | ROOM 1

18TH JUNE

PLENARY SESSION | ROOM 1

16:00H 17:10H

CAR-T CELLS THERAPY AND FLOW CYTOMETRY APPLICATION

Chair: **Alberto Órfão** (Salamanca, Spain); **José Mário Mariz** (Porto, Portugal)

16:10h - 16:55h
ADOPTIVE CELLULAR IMMUNOTHERAPIES DRIVEN BY CHIMERIC ANTIGEN RECEPTORS
Pablo Menéndez (Barcelona, Spain)

16:55h - 17:10h
Panel Discussion

PARALLEL SESSION | ROOM 1

17:10H 18:35

IMMUNOLOGY 2: IMMUNOTHERAPY

Chair: **Aberto Órfão** (Salamanca, Spain); **Artur Paiva** (Coimbra, Portugal)

17:20h - 17:50h
MONITORING CAR-T CELLS IN LYMPHOID MALIGNANCIES BY FLOW CYTOMETRY: TECHNICAL APPROACHES AND CLINICAL UTILITY
Sara Gutierrez (Salamanca, Spain)

17:50h - 18:20h
WHY DO WE NEED CAR-TS?
José Mário Mariz (Porto, Portugal)

18:20h - 18:35h
Panel Discussion

18:35h - 18:50h
BREAK | ROOM 1
(virtual commercial exhibition break)

18:50h - 19:40h
AWARD CEREMONY | ROOM 1

CLOSING CONFERENCE | ROOM 1

19:40H 20:30H

LIGHT BEYOND THE TUNNEL: CYTOMETRY AFTER HORIZON 2020

José Enrique O'Connor (Valencia, Spain)

20:30h - 20:45h
CLOSING CEREMONY | ROOM 1

14TH JUNE

PRE-CONGRESS COURSES

PCC 1. From technical aspects to automated data analysis and beyond

PCC 2. Leukaemia and lymphoma immunophenotyping: The basics

PCC 3. What you should know about immunodeficiencies: A clinical and cytometric approach

PCC 4. Nanocytometry, cell sorting and functional assays

PCC 5. Automatic analysis at diagnosis and follow-up

PCC 6. Flow cytometry in animal experimental research

PRE-CONGRESS COURSE 1

From technical aspects to automated data analysis and beyond

Carlos Palmeira
COORDINATOR
Porto, Portugal

Alfonso Blanco
COORDINATOR
Dublin, Ireland

TARGET AUDIENCE Students or clinical and research laboratory professionals, from different fields.

DESCRIPTION This course is for all professionals who wish to start using the flow cytometry in their work, what they need to know. The course provides an overview of flow cytometry, from its basic technical principles and set-up according with the best practice, to data analysis and applications in clinic and research. How to handle and simplify the analysis of large amount of data and information using automatic analysis is also illustrated and discussed in this pre-congress course.

LEARNING OBJECTIVES

› Technical concepts and principles of flow cytometry system. What is flow cytometry and what you need to know about the instrument;
› Basic rules for equipment and experiences set-up and standardization, strategies for multiparametric panel design: what to take consideration to improve panel design and choosing the right controls;
› Phenotyping and functional assays: how they can provide a more complete picture of the cell;
› Automatic analysis and data management: how to handle and analyze more data and get more information.

FLOW CONCEPTS: HOW DOES A CELL BECOME A PLOT?

Alexandre Salvador Porto, Portugal

Flow Cytometry is a technic used for the study of individual cells. Cell by cell is analyzed by the passage thru a laser. The light deflected will give us relative information on size and complexity of the cells. Also, we can add fluorochromes, so we can get more information about this cell. For example, if we add a fluorochrome that could only stain the cell if there is a damage in the membrane, like in a dead cell, we can them identify which cells are live and which are dead. Because we can also count a substantial number of cells, this measurements by flow cytometry can be statistically robust. With the new instruments available, also the number of fluorochromes that we can detect at the same time, is much higher. It´s usual to detect more of 4 at the same time. This means that we can also use antibody's, coupled with different fluorochromes, and identify, at the same time, different antigens at a cellular level. This allows us to detect co-expressions, different levels of expression, maturations and differentiations patters, etc… All this technology can, in special cases be used also to separate physically the cells (cell Sorting), even in a single cell-based assay. This could allow experiments to be possible for example, to study the expression for some gene, protein or RNA. Flow cytometry is and will be a useful tool to use in the study of your cells. The representation of the parameter, according to the detection that was made, will also be a crucial point to do interpretation. So, cells ins suspension, go in a liquid until the interrogation point, were they pass the lasers and by dispersion and emission of fluorescence, we interpret who they are. The system converts the electronics pulses created by each cell that emits signals into a digital signal, and in the end, a list mode file is created for each sample or acquisition. Only them we use software to analyze the data and interpretate the results. We can also do reanalysis or share these files with others. In resume, take in consideration:

› What instrument will you use.
› What the configuration? What lasers and filters are installed?
› What kind of sample do you have?
› Do the proper titration.
› Use the correct controls.
› Acquire enough events to do correct interpretations.
› Use a simple and correct analysis software.
› Make reproducible data.
› Control your experiment.

Flow cytometry is a powerful technology. Use it well, control and be accurate.
Everything depends in flow, but a lot depends in you!

MULTICOLOR PHENOTYPING: THE PATH TO DESIGN AND SUCCEED

Lola Martinez Madrid, Spain

Multicolor Immunophenotyping panel design should follow certain rules or principles in order to end with a robust panel that will be reproducible and will rend good quality data over time. In order to do so, you must take time to define your biological question and look in depth to the type of sample you will be running in your experiments (sample preparation is one of the optimisations to do in order to succeed in multicolor flow), the minimum number of markers you need to use in your experiment and rank them in terms of importance to identify your subset of interest and according to their level of expression in your samples. Once this is define, next will be to match those markers with the correct pair fluorochrome and for that fluorochrome brightness and spreading should be carefully look at. One other aspect that will mark your panel it is the instrument configuration available to running those samples.

Controls are also relevant when designing and testing a panel as they could be very useful to identify potential pitfalls that may impact your data resolution. If all these steps are carefully look into you will end up with a good panel.

FLOW BEYOND PHENOTYPING: PROBING CELLULAR PROCESSES USING FLOW CYTOMETRY

Timothy Bushnell New York, USA

Phenotyping, the identification of cells based on their protein expression, is the central assay in flow cytometry. It provides researchers and clinicians with a snapshot of what cells are present in normal and diseased conditions. However, phenotyping can provide so much more information when coupled with functional assays that can provide more detailed information about the state of the cell. In this lectures attendees will learn about some of these assays and how they can provide a more complete picture of the cell.

LIGHT UP YOUR RESULTS! APPLY DATA ANALYSIS AND MANAGEMENT SMARTLY

Zaida Vergara Madrid, Spain

Day after day we create more complex flow cytometry experiments to fully characterize cell populations. While this is instrumental to understand complex processes such as the immune response, after every experiment, we end up with an incredibly large amount of data. Our data sets must be analyzed, archived, shared, and rendered traceable and auditable. Find out how to leverage the Cytobank platform, a leading cloud-based solution for high dimensional data management and analysis.

Learning objectives:

› Discover how well-organized data sets are huge resources for future discoveries.
› Learn how to analyze high dimensional data with the help of machine learning algorithms to automate your analysis.
› Create meaningful and beautiful figures to better communicate your findings.

PRE-CONGRESS COURSE 2

Leukaemia and lymphoma immunophenotyping: The basics

Gabriela Martins
COORDINATOR
Porto, Portugal

Sérgio Chacim
COORDINATOR
Porto, Portugal

TARGET AUDIENCE Medical and other health science professionals with activities in relation to onco-haematology.

DESCRIPTION This course will provide a basic overview of Leukaemia and Lymphoma immunophenotyping focusing on epidemiology, clinical presentation and strategies for diagnosis mainly the immunophenotyping characterization by flow cytometry. Immunophenotyping is an essential part of the diagnostic workup and prognostic stratification of leukaemia and lymphoma and thus for an appropriate treatment of these complex and heterogeneous diseases. It provides a lot of useful information in this setting that transfers directly from laboratory to clinical management of patients.

LEARNING OBJECTIVES Epidemiology, ethology, sites of involvement and clinical features of the most frequent cases of Leukaemia and Lymphoma. Normal immunophenotyping of biological products (peripheral blood, bone marrow, lymph node aspirates, etc).
Examples of Immunophenotypic features in Various Categories of Acute Leukaemia and Mature B cell lymphoma.
Analysis of cases using proper software. Participants will have prior access to the analysis software and listmode of clinical cases that will be discussed during the course.

EPIDEMIOLOGICAL ASPECTS FOR LYMPHOMA AND LEUKAEMIA, ITS CLINICAL PRESENTATION AND STRATEGIES FOR DIAGNOSIS

Sérgio Chacim Porto, Portugal

Clinical lymphoproliferative diseases present themselves with different epidemiological aspects. This talk will address lymphocyte development in order to understand lymphoid malignancies classifications (B and T -cell differentiation within human body). We will talk about known risk factors in developing Non Hodgkin lymphoma, as well as common clinical presentation of this disease. There will be a focus on diagnosis, with all its morphology aspects, Immunophenotyping and molecular genetics and cytogenetics. Staging and prognostic aspects will be focused.

We will discuss acute myeloid leukemia as a clinical model for acute lymproliferative diseases, approaching its different aspects for diagnosing, in order to classify it, according to recent publications. The initial working panel for evaluating a patient and treatment hallmarks will be discussed. The new and hot-topic refractory disease option treatments will later be revealed, its mechanisms of action and efficacy and toxicity.

ACUTE LEUKAEMIA: IMMUNOPHENOTYPIC ANALYSIS AND INTERPRETATION

Carlos Fernández Giménez Salamanca, Spain

Leukemia is one of the most common malignant disorders affecting the world population, mainly in more developed areas. Globally, in 2018, leukemia ranked as the fifteenth most common diagnosed cancer with 437,033 cases and 309,006 mortalities, amounting to the eleventh cause of death due to malignant disorders. However, the geographic distribution of leukemia is universal, with higher prevalence and overall mortality in the more developed countries, probably caused because of the improvements in quality of life. The mortality rate, however, is higher in developing countries where public health systems, access to therapies and care of patients remains as a challenge.

Acute leukemias (AL) are malignant clonal disorders of blood-forming organs involving one or more cell-lines in the hematopoietic system. These disorders are marked by the diffuse replacement of bone marrow with abnormal immature and undifferentiated hematopoietic cells, resulting in reduced numbers of erythrocytes and platelets in the peripheral blood. Based on the origin of the abnormal hematopoietic cells involved, such as lymphoid, myeloid, mixed or undifferentiated, these disorders are classified accordingly. This classification has been modified according to the access to the knowledge about the pathophysiology of these diseases. In fact, first classifications were based on cytomorphology; this was a very important step, but not good enough. The irruption of molecular and immunohistochemistry approaching had a tremendous impact; pathophysiology was much better understandable and therefore, outcome, prognosis and predictor factors were proposed, basically based on the genetics, improving the stratification of patients in order to propose the best therapy options and opening new perspectives for new drugs and treatments (less aggressive and more efficient).

In parallel, the development and improvement in immunophenotyping, with new technology, more robust knowledge about the NORMAL hematopoiesis and the abnormal patterns detected in patients with acute leukemia, in many cases correlated with specific genetic aberrancies. These facts claimed the need of a new landscape for flow cytometry and hematology, where all the benefits of this technique could improve dramatically in the best knowledge of the normal and clonal hematopoiesis, providing physicians robust information useful for diagnosis, prognosis and monitoring of these diseases.

The improvements started with the standardization of technique. Then with the development of useful panels built in order to answer key clinical questions: lineage of blast, phenotypic profiles related to prognosis and outcome for patients and further monitoring.

In the last years main advances have come specially in BCP-ALL where flow cytometry provides a high robust information about the kinetic of the disease, a new dynamic approaching about the leukemic transformation and evolution and immunophenotypic profiles related to recurrent cytogenetic changes with prognostic impact.

Unfortunately, in TCP-ALL, the immunophenotyping shows less impact due to till to date, no changes in the phenotype are related to specific molecular(genetic) changes. However, flow cytometry provides useful information to classify and stratify at least cortical T AL and shows the high specificity to identify the Early T AL. Of course, a long tryp remains to be done and we hope in short/medium time improvements will be available.

Finally, flow cytometry has shown the utility and application in myeloid hematopoiesis. The development of standardized panels focus on the study of myelopoiesis from a dynamic point of view has changed our minds about the clonal myeloid hematopoiesis focused on MDS and AML. Thus, AML immunophenotyping allows to give important and strong answers about the blasts in parallel with the microenvironment; a better discrimination among the blast, their lineage and degree commitment, detection and characterization of multiple clones, crucial to predict the outcome and potential response and relapse. In these terms, flow cytometry has shown the utility specially in promyelocytic acute leukemia, undifferentiated leukemia, mixed phenotype AL, blastic plasmacytoid dendritic cell neoplasm.

In this pre congress course, our main goal is to provide the attendants tools for a better understanding about new and modern analysis strategies. These are based on well-structured profiles/templates in order to facilitate the work

and rationale interpretation in line to report properly, answering clinical and biological questions that might improve the quality of the diagnosis as well as the clinical stratification and monitorization.

MATURE B CELL LYMPHOMAS: IMMUNOPHENOTYPIC ANALYSIS AND INTERPRETATION

Joana Caetano Lisbon, Portugal

- Overview on the role of flow cytometry in the diagnosis of mature B-neoplasms
- B-cell differentiation and correspondence of B-cell neoplasms
- Normal B-cell phenotype
- Strategy for diagnosis of mature B-cell neoplasm
- Euroflow approach to screening and characterization of mature B-cell neoplasms
- Antigen expression levels in normal versus B-cell neoplasms
- Analysis and interpretation of typical mature B-cell neoplasms

PRE-CONGRESS COURSE 3

What you should know about immunodeficiencies: A clinical and cytometric approach

Esmeralda Neves
COORDINATOR
Porto, Portugal

António Marinho
COORDINATOR
Porto, Portugal

TARGET AUDIENCE Medical and other health science professionals with activities in relation to immunology.

DESCRIPTION Flow cytometry is a highly sensitive tool for evaluating the immune system and supporting the diagnosis of Primary Immunodeficiency (PID). The applications of flow cytometry in the evaluation of PIDs are multiplex and include the investigation of specific cell populations and subpopulations, specific cell membrane, intracellular and intranuclear proteins, biologic effects associated with immune defects, and functional immune abnormalities.

LEARNING OBJECTIVES Epidemiology, etiology, sites of involvement and clinical features of Primary Immunodeficiencies. Immunophenotyping of normal lymphoid subpopulations in peripheral blood. Examples of Primary Immunodeficiencies – Analysis of cases using the proper software.

AN INTRODUCTION TO INBORN ERRORS OF IMMUNITY

Cátia Iracema Morais Porto, Portugal

Inborn errors of immunity (IEI) are a heterogeneous group of diseases that result from the impairment of normal immune development or function. Albeit individually rare, IEI have a group prevalence up to 5 in 1000.
Clinical presentation is diverse and includes recurrent, severe and/or unusual infections, autoimmune features, allergy and an increased susceptibility to malignancy.

The cornerstone of a thorough approach to a possible IEI is a good clinical history and physical examination. From there, if warning signs of IEI are identified, screening lab tests should be ordered, including parameters that allow for exclusion of relevant differentials. With this information, it is sometimes possible to identify the most likely involved element of the immune system.

Further laboratory evaluation should be done in a stepwise approach, with increasing complexity, leaving more differentiated analysis for the referral specialist. Definitive diagnosis relies on identification of the causative genetic defect, which allows for an adequate choice of treatment.

During this presentation, we will review the most common presenting features and screening laboratory findings of the ten categories of IEI according to the International Union of Immunological Societies' classification, and briefly address the approach to treatment of these disorders.

WHAT YOU SHOULD KNOW ABOUT IMMUNODEFICIENCIES: A CLINICAL AND CYTOMETRIC APPROACH

Martin Perez Andrés Salamanca, Spain

The participants will learn the critical parameters that are required for diagnostic and classification of primary immunodeficiencies (PID) by flow cytometry including: 1) recommended parameters to evaluate in PID patients according to international guidelines (ESID, WHO, IUIS), protocols and techniques required for the analysis of these parameters, and reference values.

EMERGENCIES ASSOCIATED TO INBORN ERRORS OF IMMUNITY: THE ROLE OF THE FLOW CYTOMETRY WITH PRACTICAL CASE ANALYSIS

Catarina Martins Lisbon, Portugal

Inborn Errors of Immunity (IEI), previously known as Primary Immunodeficiency disorders, are a diverse and growing group of diseases, with recognized defects or functional impairment in immune players, often identified in early childhood and adolescence. In some scenarios, Inborn Errors of Immunity can arise as paediatric emergencies, as it happens with SCID or hemophagocytic syndromes.

In these situations, a timely identification of the underlaying condition is crucial, and flow cytometry represents an important laboratory methodology with the necessary promptitude and effectiveness. Thus, clinicians and laboratory scientists need to properly identify and recognize the flow cytometry tests helpful for the diagnostic of IEI in diverse situations, and obviously interpret their respective alterations, such as the typical lymphocyte immunophenotypic profile in SCID patients, or the characteristic features of functional flow cytometry assays in disorders like CGD or hemophagocytic syndromes. Moreover, flow cytometry can bring relevant data for distinguishing primary and secondary causes, as happens in primary and secondary hemophagocytic syndromes.

With this talk we aim to explore flow cytometry approaches in the context of IEI, particularly applicable to emergency

situations, exploring clinical cases of IEI patients assessed with flow cytometry-based assays.

In sum, Flow Cytometry is a crucial tool for both diagnostic and monitoring purposes in patients with IEIs, assuring a diversity of immunophenotyping and functional assays that can be applied in a sequential manner. Besides, when considering patients with severe presentations of these diseases that need prompt and adequate medical decisions, Flow Cytometry provides fast and reliable results, many times when no other confirmatory tests are available. Furthermore, these results can orientate diagnostics and genetic studies, and though there is still room for improvement, the application of standardized strategies that monitor the evolution of the immune functions is also a key element in the follow-up of patients with distinct immune defects.

CLINICAL AND LABORATORY DIAGNOSTIC APPROACH OF COMMON VARIABLE IMMUNODEFICIENCY (CVID)

Kissy Annet Guevara-Hoyer Madrid, Spain

Common variable immunodeficiency is the most frequent symptomatic primary immunodeficiency. It is characterized by an increased predisposition to recurrent bacterial infections, resulting from low antibody production. A wide range of manifestations including increased risk of autoimmunity, enteropathy, inflammatory diseases, polyclonal lymphocytic infiltration and malignancy are associated with CVID. CVID seems to result from various factors that contribute to a defect in antibody production, existing epigenetic, and genetic factors that may be involved. Despite years of research, the precise pathogenetic mechanism of CVID remain unknown. Among the main alterations described in CVID are defects in the differentiation stages of the B lymphocyte, alteration in T-B costimulation signals, defects in dendritic cells, monocytes/macrophages, NK cells, as well as markedly reduced levels of serum immunoglobulin free light chains. Genetic analysis of CVID is essential in the differential diagnosis and precision medicine approach. This would be especially relevant in CVID patients with a severe phenotype. In most cases, CVID occurs sporadically. Family inheritance represents 10% of them. Dominant inheritance, autosomal patterns with variable penetrance, autosomal recessive, and linked to X chromosome have been described.

As future projections, it should be considered that the combination of several diagnostic biomarkers (genetic, biochemical, and immunological) and the development of predictive scores would allow a better classification and, therefore, a better clinical approach from CVID patients.

PRE-CONGRESS COURSE 4

Nanocytometry, cell sorting and functional assays

Artur Paiva
COORDINATOR
Coimbra, Portugal

Bruno Costa Silva
COORDINATOR
Lisbon, Portugal

TARGET AUDIENCE Students or clinical and research laboratory professionals, from different fields.

DESCRIPTION Despite recent advances, the study and application of “small particles”, such as, microparticles, extracellular vesicles, or viruses, in basic and clinical research, remains a major challenge due to the lack of standardized protocols. Flow cytometry has been suggested as a suitable technology for their detection, sorting and analysis. But how to do it? What technical and practical aspects should be considered, from cytometer set up to analysis?How to validate results? What kind of applications at structural and functional level can be used? These are some of the topics covered and discussed throughout this pre-congress course.

LEARNING OBJECTIVES

- Small particles: concepts and biology; what is the interest?
- Sorting in nanocytometry: why and how to perform it? Good practice guidelines
- Cytomics assays applied to small particles study: validation, potential in basic as well as in clinical research

INTRO TO SMALL PARTICLE DETECTION USING FLOW CYTOMETRY

Rui Gardner New York, USA

The importance of detecting sub-micron particles in flow cytometry has become extremely relevant in the last decades given the role of viruses and extracellular vesicles in health and disease, as well as the use of nanoparticles for drug delivery and therapeutics. In this first presentation, we will introduce some of the basic concepts and best practices to measure sub-micron particles in flow cytometry.

HOW TO APPROACH SORTING OF BIONANOPARTICLES. VIRUSES AND EXTRACELLULAR VESICLES

Òscar Fornas Barcelona, Spain

Nanoparticle sorting is one of the most complex applications of flow cytometry. Basically, because we are very close to or below the resolution limit of the technique. Precisely for this reason, unlike large-particle applications, we must use other technologies that validate our methodology. Without such validations, we cannot fully believe the data we obtain by cytometry. Huge amount of published data of nanoparticles analysis by flow cytometry generate interesting discussions but unfortunately a methodological consensus has not yet been reached. With this presentation we intend to show our approach to the study of nanoparticles using flow cytometry, through our experience handling nanoparticle, as well as the steps we have followed to validate the analysis and sorting of these nanometric particles.

FUNCTIONAL ANALYSIS OF SUBCELLULAR COMPONENTS AND EXTRACELLULAR VESICLES BY CYTOMICS

José Enrique O'Connor Valencia, Spain

This presentation is intended to integrate flow cytometry as a choice methodology among the current techniques for studying extracellular vesicles, as well as to provide a panoramic view of the evolution and most relevant current applications of flow cytometry in this growing field. While the clinical applications of flow cytometry to extracellular vesicles were mostly oriented to clinical purposes, early work with isolated mitochondria and isolated Golgi vesicles showed the feasibility of applying conventional flow cytometers to functional characterization of isolated subcellular microparticles. Because of the current interest in exosomes and other extracellular vesicles, basic, translational and clinical studies by flow cytometry are of paramount relevance, and reports on technical improvements and specific working guidelines have been published, which help to standardize a complicated technical and biological issue.

Current applications of cytometric analysis of extracellular microvesicles in basic biology include the detection of extracellular vesicles and microparticles, the phenotypic and functional characterization of extracellular vesicles and the sorting of extracellular microparticles for –omic studies. Translational and clinical applications include the identification of extracellular vesicles as signaling factors, the establishment of extracellular vesicles as disease biomarkers and risk factors, as well as the functional characterization of exosomes as drug-delivering microparticles. Finally, the recent strategies for microvesicle analysis based on direct capture of exosomes on different types of beads, allowing easy exosome purification, but also exosome phenotyping and quantitation by flow cytometry, as well as the application of bioinformatic tools to multiplex assay of exosomes.

PRE-CONGRESS COURSE 5

Automatic analysis at diagnosis and follow-up

José Carlos Segovia
COORDINATOR
Madrid, Spain

Maria Jorge Arroz
COORDINATOR
Lisbon, Portugal

TARGET AUDIENCE Flow cytometry users in clinical laboratories.

DESCRIPTION The continuous technological development of flow cytometry, both in terms of equipment and phenotypic and functional markers, has provided a greater ability to identify and characterize a growing number of cell populations. This has important implications for diagnosis and minimal residual disease (MRD) flow cytometry applications in hemato-oncology. However, processing and analysing such a growing volume of data using conventional data analysis software, is an increasingly difficult task. Thus, advanced analysis tools capable of supporting flow cytometry users to process all this information have become precious means in clinical laboratories. In addition to its clinical value, automatic analysis makes way for greater standardization and reduces intra and inter-laboratory variability in the analysis process.

LEARNING OBJECTIVES Participants will gain a deep understanding of available informatic tools to support flow cytometry data analysis and they will increase their knowledge and experience in advanced software-assisted analysis and reporting of flow cytometry data using these tools, in acute leukemia and chronic lymphoproliferative disorders both at diagnosis and follow-up. Participants will have prior access to the analysis software and listmode of clinical cases that will be discussed during the course.

THE USE OF REFERENCE DATABASES IN AUTOMATIC ANALYSIS: THEORETICAL BASES

Ana Bento Salamanca, Spain

In this presentation, Ana Catarina Bento will highlight how to increase the accuracy and precision of data analysis results in the diagnosis, monitoring and follow up of hematological diseases, through the EuroFlow™ algorithms and databases as a new way of data analysis. For this purpose, she will explore the theoretical bases necessary for a better understanding of the algorithms included in the automatic classification, contributing also for a better comprehension of data evaluation, crucial to complete the EuroFlow™ standardization approach.

APPLICATIONS OF AUTOMATIC ANALYSIS IN THE SCREENING OF CHRONIC LYMPHOPROLIFERATIVE NEOPLASIA AND ACUTE LEUKAEMIA; ANALYSIS OF CLINICAL CASES

Paula Fernández Aarau, Switzerland

The lecture will give you an introduction to the process of building the LST and ALOT databases by the EuroFlow consortium, the quality control mechanism those files underwent as well as the validation of the automated gating and interpretation tool against manual analysis. The use of the database tool will be illustrated in detail using case files.

APPLICATIONS OF AUTOMATIC ANALYSIS IN MRD TESTING IN B-ALL AND MULTIPLE MYELOMA; ANALYSIS OF CLINICAL CASES

Juan Flores Montero Salamanca, Spain

The process of complete standardization of flow cytometry MRD assessment also includes the stage of data analysis and interpretation. Software assisted data analysis tools based on reference databases are being more and more incorporated on routinary use. These tools have demonstrated to be highly sensitive, hold a convenient cost-effective balance, and maintain a very high correlation when compared vs fully expert-based analysis strategies, which makes them suitable to be used on residual disease detection. During the presentation, we will review the particularities of automated gating and identification tools in the field of residual disease determination and follow the analysis of practical, real-life B-ALL and MM samples to illustrate its utility.

PRE-CONGRESS COURSE 6

Flow cytometry in animal experimental research

Rita Ferreira
COORDINATOR
Aveiro, Portugal

Fátima Gärtner
COORDINATOR
Porto, Portugal

TARGET AUDIENCE Students and researchers from different fields.

DESCRIPTION The course not only addresses the main concepts of model design experiments in animal studies but explains how researchers can apply flow cytometric methods to answer their research questions, namely immunological characterization and selection of an appropriate pre-clinical model.

LEARNING OBJECTIVES

- Animal experimental models for the study of human diseases: how to execute, criteria for their choice and monitoring;
- Sample preparation: how to optimize sample preparation to ensure viability of your cells;
- Multi-colour antibody panel design: how to optimize How to design an effective multi-colour antibody panel and how to troubleshoot your data;
- Immune cell subpopulations in tumor models: how study them by flow cytometry;
- Mice as a model to study the immune system;
- Flow cytometry as a tool for the characterization of mouse thymic cells.

THE USE OF ANIMAL MODELS IN MEDICAL RESEARCH: FROM THE LEGAL ASPECTS TO ITS PRACTICAL EXECUTION

Paula Oliveira Vila Real, Portugal

The concern with laboratory animals has intensified since the 3Rs publication by Russell and Burch, in 1959. In this sense, there is a growing preoccupation by the European Community, and each of its member states, to disseminate among their researchers the application of 3Rs in all experimental works that involve animals. In order to standardize

its applications, it is fundamental to know the national legislation, extrapolated from the European legislation. This oral presentation will explain the Portuguese legal aspects necessary to obtain authorization for the use of animals for scientific purposes, based on the realization of some animal models.

USING AN INTEGRATED SOP APPROACH TO DESIGN FLOW CYTOMETRY EXPERIMENTS FOR ANIMAL RESEARCH

Jane Srivastava California, USA

'When designing a multi-colour antibody panel for a flow cytometry experiment, there are many considerations to think about other than just what fluorophore to assign to what antibody. Several factors contribute to a successful experiment outside of panel design, including background research, sample preparation and data analysis. Using a model of designing a 15 colour panel for determining mouse bone marrow mesenchymal stromal cell populations, this presentation shows how combining different aspects of experimental design into a consolidated standard operating procedure can reduce ambiguity and lead to a reliable and strong template to utilise in flow cytometry experiments.'

DISSECTING THE DIVERSITY OF MOUSE THYMIC CELLS BY FLOW CYTOMETRY

Pedro Ferreirinha Porto, Portugal

The immune system, composed by different effector cell populations, is an essential part of our body's defence against invading pathogens and cancer cells. The development of these different cell types is the result of a complex network of differentiation processes that occur in primary lymphoid organs. The thymus, containing thymic epithelial cells (TECs), is the primary lymphoid organ required for the development of T cells. However, its unique biology imposes limitations that make the in-depth study of this organ in humans challenging. Over the decades, mice have emerged as the preferred animal model to study the immune system due to the unique advantages of this species, as well as close parallelisms with the human immune system. In this work, using mice and flow cytometry, we tackle the inherent diversity of medullary thymic epithelial cells (mTECs), a key population for the development of functional but self-tolerant T cells. Of notice, we develop a novel flow cytometry panel to dissect mTECs through the inclusion of the markers CD24 and SCA1. Their analysis combined with markers previously established for mTEC assessment provides an efficient strategy to dissect different mTEC subpopulations and map the final stages of differentiation of this critical cell population for tolerance induction.

PROFILING OF IMMUNE-ONCOLOGY AGENTS IN SYNGENEIC TUMOUR MODELS

Lukasz Magiera Cambridge, UK

Breakthrough in cancer therapy caused by the discovery of immune checkpoint blocking antibodies such as αPD-1, αPD-L1, and αCTLA-4 reinvigorated interest in understanding and development of novel immunotherapy agents. Mouse syngeneic models, which have a functional immune system, represent an essential tool for pre-clinical evaluation of new immunotherapies. However, immune response varies widely between the available models and the translational relevance of each of them is not fully understood, making selection of an appropriate pre-clinical model for drug development challenging. Therefore, it is essential to understand the dynamic interplay between the tumour and the immune system and develop robust methodology to enable high-volume drug development studies. We used some of the most established syngeneic models, such as CT-26, MC38 and 4T1, to characterise their microenvironment and changes in immune populations that occur over time. We then applied those findings to explain the underlying differences in their responses to immune checkpoint blockade and guide model selection for development of novel immunotherapies.

15TH JUNE

CONGRESS

Monitoring of therapy in hematological malignancies: Can we move from bone marrow to blood?

Hematology 1: Advances in diagnosis (Part 1)

Microbiology: Clinical and flow cytometry application

Hematology 1: Advances in diagnosis (Part 2)

GECLID MEETING

OPENING CONFERENCE | ROOM 1

Monitoring of therapy in hematological malignancies: Can we move from bone marrow to blood?

Alberto Orfão Salamanca, Spain

Since the late 1990's monitoring of minimal residual disease (MRD) by either PCR-based molecular approaches or multicolor flow cytometry has proven to be of great clinical utility in most acute and chronic leukemias. Thus, due to its higher sensitivity in detecting residual tumor cells, MRD measurements provide an in-depth assessment of the quality of conventional (complete) response to therapy, at the same time it has emerged as (one of) the most valuable prognostic factor(s), independently of the therapy administered. Because of this, MRD has progressively been included in prospective clinical trials and therapeutical protocols in e.g., childhood and adult acute lymphoblastic leukemia (ALL) and multiple myeloma (MM), among other hematological malignancies. In such studies, assessment of MRD levels in bone marrow (BM) at specific time points after starting therapy, has been used for both re-stratification of patient risk and MRD-directed treatment intensification and to a less extent also, de-escalation leading to significantly improved patient outcomes. Due to such important clinical utility, and progressive adoption of BM MRD testing in many centers worldwide, selection of the most adequate and robust MRD assay has become of utmost relevance.

At present it is well-established that together with PCR-based next-generation sequencing, next generation flow cytometry (NGF) techniques are the preferred methods for MRD monitoring in leukemia and MM. Compared to conventional flow cytometry, NGF approaches are typically based on i) optimized and validated multi-color (>8-color) antibody panels, that include a set of markers for high-sensitive and specific detection of tumor cells and simultaneous estimation of sample hemodilution, ii) acquisition of high numbers of BM cells (i.e. >10 million cells/sample), and iii) automated gating, data analysis and reporting for an improved reproducibility. The standardized and validated EuroFlow NGF panels and procedures are particularly suited for standardized MRD monitoring in e.g., ALL and MM. This is mainly due to the fact that their performance has been technically validated against the ASOqPCR gold standard or high-sensitive NGS approaches, and clinically tested in large prospective cohorts of uniformly treated patients.

Despite all the above advantages and contributions, MRD monitoring in BM remains a suboptimal procedure for monitoring therapy in most hematological malignancies. This relates to the fact that BM aspiration remains an invasive procedure that cannot be frequently repeated in the same patient, particularly in children and in elderly patients; moreover, BM samples are usually diluted with variable levels of blood, and frequently their analysis does not provide an accurate, and sometimes even representative, estimation of the tumor burden in BM due to a heterogeneous (patchy) pattern of infiltration by the tumor, affected also by the relative counts associated with BM aplasia vs regeneration. In order to overcome some of these limitations, in recent years, monitoring of circulating tumor cells (CTC) in blood has become a matter of investigation. A major advantage of monitoring CTC in blood vs BM MRD is that blood sampling is a minimally invasive procedure suitable for more frequently monitoring, it provides absolute (as well as relative) tumor cell counts, it more closely reflects tumor dissemination, and allows for simultaneous CTC and immune monitoring, particularly in the setting of patients treated with novel immunomodulatory and immunotherapeutic agents. Of note, the increased sensitivity reached with the novel NGF approaches developed for BM MRD monitoring, has set the basis for investigation of their utility for CTC detection both at diagnosis and after therapy, particularly in patients with e.g., ALL, acute myeloblastic leukemia (AML), and MM, among other hematological malignancies.

Early CTC studies performed in blood samples of ALL patients, already showed a high degree of agreement between CTC levels in blood and BM MRD in T-ALL. In contrast, the same studies revealed that in most B-cell precursor (BCP) ALL patients in whom the BM was MRD+, no CTC were detectable in blood. With the increase in sensitivity of both molecular and flow cytometry approaches, more recent studies have been developed in the last three years. These studies have confirmed the previously reported similar rates of positivity for CTC in blood and MRD in BM in patients with T-ALL. In addition, these studies also showed that in BCP-ALL, the increased sensitivity of NGS and NGF is associated with a significant decrease in the frequency of discrepant cases (from 75% to 30% of positive samples). Similar results to those observed in T-ALL have also been reported in AML by several groups in independent patient cohorts. In addition, recent studies in MM also proved that CTC are detectable by NGF at diagnosis in blood of virtually every smoldering MM and symptomatic MM patient; in around one fourth of cases persistent CTC in blood are also detectable after therapy, including around 15-20% of patients who achived complete response. Importantly, studies comparing the clinical impact of the persistence/re-emergence of CTC in blood vs BM MRD have shown a complementary prognostic value for both assay measurements. Thus, despite virtually all ALL, AML and MM patients that show CTC in blood after therapy are also MRD+ in BM, representing only a fraction of all BM MRD-positive cases, the presence of CTC in blood identifies a subgroup of BM MRD+ patients at higher risk of relapse/disease progression, who might potentially benefit from earlier treatment interventions. In addition, it permits simultaneous monitoring of persistence of CTC in blood and disease response to distinct modalities of immunotherapy. As an example, protocols have been developed for simultaneous monitoring of up to hundreds of different subsets of CART cells and other immune cells, in addition to CTC, in patients who had received CART cell-based treatments.

In summary, in the last decades MRD monitoring has been recognized as one of the most valuable biomarkers for in-depth analysis of response to therapy, re-stratification of patient risk and early treatment intervention in the setting of large multicentric protocols and clinical trials. Despite all advantages and the clinical value MRD monitoring in BM is associated with several practical limitations that have fostered the feasibility and clinical utility of blood-based monitoring of CTC vs BM MRD. Preliminary data indicates that sequential monitoring of CTC in blood provides an attractive and clinically informative tool for more frequent monitoring of response to therapy in patients with hematological malignancies, including ALL, AML and MM, allowing both identification of BM MRD+ patients who might benefit from earlier treatment interventions and simultaneous CTC and immune monitoring in the settings of immunotherapy.

PARALLEL SESSION | ROOM 1

Hematology 1: Advances in diagnosis (Part 1)

Chair: **Margarida Lima** Porto, Portugal; **Rui Henrique** Porto, Portugal

IMMUNOPHENOTYPIC IDENTIFICATION OF SÉZARY CELLS IN BLOOD USING EUROFLOW STRATEGIES

Julia Almeida Salamanca, Spain

Sézary syndrome (SS) is defined by the clinical triad of erythroderma, generalized lymphadenopathy and the presence of neoplastic, clonally related, T cells in peripheral blood (PB) and skin. The diagnosis of SS is often challenging, due to non-specific clinical and histopathological features that are also frequently seen in much more prevalent benign erythrodermic skin disorders. In such circumstances, the unequivocal identification of Sézary cells and the assessment of tumor burden in PB is crucial for diagnosis, and becomes progressively relevant for disease staging and treatment monitoring. Flow cytometry is considered the ideal method for detecting Sézary cells, which in clinical practice is mostly based on the characteristic loss of CD26 and CD7 surface molecules by tumor cells, usually associated to lower expression of other pan-T-cell markers. However, it should be noted that there is no specific marker for Sézary cells, as normal $CD4^+$ T cells (both in basal and particularly in reactive conditions) may show phenotypes that resemble that of Sézary cells (i.e. a $CD7^-CD26^-$ profile), mainly when flow cytometry analysis is based on single or individual markers. More recent studies have described expression of CD279 (PD-1), killer-cell immunoglobulin-like receptors (particularly CD158k -KIR3DL2-), and the CD164 adhesion molecule or the actin-bundling protein T-plastin on Sézary cells, but the percentage of SS cases found to express these markers is variable among the different studies and usually not all SS cells in positive cases express them; additionally, the expression of CD158k and particularly CD164 has not been adequately analyzed in normal $CD4^+$ T cells, to identify overlapping phenotypes. On the other hand, it is well-known that Sézary cells may show heterogeneous

phenotypes other that the most frequent and typical immunophenotypic profile (i.e. CD3/TCRαβ^{lo}, CD28$^{+/++}$, CD4^{lo}, CD2$^{-/lo}$, CD7$^{-/lo}$ and CD26$^{-/lo}$); therefore, tumor cells can vary from patient to patient, and usually even in the same patient, particularly after treatment. In addition, certain phenotypic features of Sézary cells can be also observed in T-cell chronic lymphoproliferative disorders other than SS or mycosis fungoides. Accordingly, tumor cells from erythrodermic adult T-cell leukemia/lymphoma, and even from T-cell prolymphocytic leukemia (that can also rarely present with erythroderma and in some cases with the cells showing low expression of CD3 and absent CD7) may have a similar phenotype as Sézary cells, as well as many peripheral T-cell lymphomas, not otherwise specified.

To address these issues and try to solve all the pitfalls associated to the immunophenotypic diagnosis of SS, within the EuroFlow Consortium and since 2019 in collaboration with the *EORTC Cutaneous Lymphoma Task Force*, we applied highly sensitive and standardized EuroFlow-based multiparameter flow cytometry methods and tools, to specifically identify Sezary cells and define in detail the immunophenotype of tumor cells, to distinguish them from their normal cell counterpart(s). For this purpose, it is crucial to precisely dissect the phenotype of normal CD4^{+} T cells (in basal and reactive conditions) stained with the same antibody panels -as the reference for the identification of aberrant/clonal CD4^{+} T cells-, and to focus on the identification of aberrant cells based on the whole phenotypic parameters. For these purposes, we included "classical" markers (i.e. CD2, CD3, CD4, CD7, CD8, CD26, CD28, CD45) with maturation-associated molecules (i.e. CD45RA and CD27, among others) to distinguish aberrant from normal CD4^{+} T cells within the corresponding maturation stage. Using these strategies and based on a detailed knowledge of the immunophenotypic features of normal memory CD4^{+} T cells, Sézary cells could be identified in most SS cases (>90%); dilution experiments (of aberrant cells with progressively higher numbers of normal CD4^{+} T cells) showed that Sézary cells could be detected at a sensitivity level up to 10^{-4}. Therefore, our preliminary results showed that EuroFlow-based standardized flow cytometry approaches allows the detection of Sezary cells with (relatively) high specificity and sensitivity. Nevertheless, in some cases a variable degree of overlapping with normal CD4^{+} T cells still existed, so we further proposed to include additional novel markers (i.e. CD158k, CD194 and CD279) and the anti-TCR-Cβ1 antibody reagent -recently available to assess T-cell clonality by flow cytometry-. The additional value of these novel markers for a more accurate identification of Sézary cells using EuroFlow-based strategies and tools are currently being investigated and validated in multicenter setting.

APPLICATIONS OF FLOW CYTOMETRY TO THE DIAGNOSIS OF HEREDITARY PLATELET DISORDERS

Catarina Lau Porto, Portugal

Inherited platelet function disorders are a very heterogeneous group of diseases, which makes the diagnosis a challenge. In the last few years, there has been a great advance in the knowledge of new genetic variants. This knowledge has shown that an accurate diagnosis has prognostic importance, since certain entities are associated with the predisposition to malignant hematological diseases (RUNX1-related thrombocytopenia, ANKRD26-related thrombocytopenia, and ETV6- related thrombocytopenia), bone marrow failure syndromes (MPL and MECOM mutations) and syndromic diseases (MYH9-related thrombocytopenia).

In our point of view, the diagnostic approach to congenital platelet diseases should follow a stepwise algorithm. This strategy is particular important and essential to guide the genetic study and to help in its interpretation, especially when carried out by next-generation sequencing (NGS) techniques, that needs to be critically evaluated taking into account clinical and laboratory phenotypes. The first steps include platelet count, peripheral blood smear, platelet function analyzer (PFA), light transmission aggregometry and flow cytometry studies.

Contributions of flow cytometry comprise simple tests like the confirmation of platelet count in select cases (ex. giant platelets) and platelet size evaluation using forward scatter (FSC). Cytometry also allows the study of platelet membrane receptors with important functions, such as the fibrinogen receptor (GpIIb-IIIa), the von Willebrand factor receptor (GpIb-X-IX), the collagen receptors (GpIa-IIa and GpVI), among others. As mutations in GFI1B gene that cause macrothrombocytopenia have been associated with increased expression of CD34 in platelets, this assessment has been proposed as a screening test for GFI1B mutations. Finally, several studies of platelet activation can be performed by flow cytometry, using different agonists and evaluating the expression of membrane molecules only expressed after activation, such as the GPIIb-IIIa activation epitope (PAC1), bound-fibrinogen, P-selectin (released from α-granules), among others. This allows for the measurement of platelet activation at basal conditions and their reactivity in response to various agonists.

Microbiology: Clinical and flow cytometry application

Chair: José Enrique O'Connor Valencia, Spain; **Rui Medeiros** Porto, Portugal

FLOW CYTOMETRY ON CLINICAL MICROBIOLOGY LAB. WHY NOT?

Cidália Pina Vaz Porto, Portugal

Flow cytometry is a multiparametric study of cells, usually human cells. Microbiology classic tests have long time-to-result and are blind regarding cell characteristics. Several applications of flow cytometry to Microbiology were developed namely regarding yeasts, parasites, Mycobacteria and Legionella both regarding its detection and antimicrobial susceptibility with great accuracy. Antimicrobial resistance (AMR) is a pandemic parallel and interacting to COVID-19. An increase of AMR is expected with COVID-19 pandemic and lessons regarding global action plans should be undertaken. Rapid AST urged and FASTinov, a spin-off of Porto University, developed a flow cytometric antimicrobial susceptibility test (FAST) directly from positive blood cultures shorting the time-to-result from 2 days to 2 hours. An external validation in a reference hospital in Madrid had just finalized and the CE mark obtained. New projects regarding the study of other microorganisms and other drugs are on-going as well as a new project using flow cytometry to quantify the active amount of antibiotics on patient´s samples. Flow cytometry has a great potential in Microbiology and a similar impact, to what happened in Hematology and Immunology, is expected.

EVALUATION OF AN ULTRA-RAPID FLOW CYTOMETRIC ANTIMICROBIAL ASSAY DIRECTLY FROM BLOOD CULTURES

Ana Dias Porto, Portugal

Bloodstream infections (BSIs) remain a major public health concern with high rates of morbidity and mortality. Antimicrobial susceptibility testing (AST) of microorganisms causing BSIs is crucial for optimal antimicrobial therapy. Current conventional methods require sub-culture of positive blood cultures (BCs) to begin AST, taking nearly 2 days for definitive results. New approaches avoiding the sub-culture step can reduce time-to-result but, are still growth-based taking at least 6 hours to report. A rapid AST is of critical importance once it could target the adequate therapy, decrease the misuse of drugs and even more importantly avoid clinical failure and thereby reducing morbidity and mortality.

FASTinov® kits uses a disruptive technology for direct ultra-rapid AST of Gram-negative and Gram-positive bacteria in BSIs showing time-to-result of 2 hours versus nearly 2 days with current methodology. FASTinov technology uses panels where the main drugs active against Gram-negative and Gram-positive organisms are present, in combination with fluorescent dyes. After one hour incubation this panels are analyzed by flow Cytometry, and using a software developed by FASTinov, it is possible to determine the antimicrobial susceptibility of bacteria's present in the BC. This is not a growth dependent method, but a phenotypic-functional study of the microorganisms' metabolic response after drug exposure using Flow cytometry. vPerformance of FASTinov® kits were evaluated in two kits, one for Gram-negative (Enterobacterales, Pseudomonas and Acinetobacter) and another for Gram-positive (Staphylococcus and Enterococcus) organisms by testing primary drugs used to treat sepsis. In parallel a proof-of-concept in an external clinical environment was performed at Ramon y Cajal hospital in Madrid (Spain), using patients' BC. A total of 175 positive BC (60 Gram-negative and 115 Gram-positive bacteria) were included with 85 patient samples (39 Gram-negative and 46 Gram-positive). After extracting microorganisms from positive BC, bacteria were incubated for 1 hour at 37ºC with antibiotics together with fluorescent probes followed by flow cytometric analysis with CytoFLEX (Beckman Coulter) platform. Both CLSI and EUCAST criteria were followed and a dedicated software used to produce a report. The obtained phenotype, as well as a screening test for a possible presence of a carbapenemase production and AmpC production in case of a group I Enterobacterales (E. coli, Kl. pneumoniae, Proteus spp, Salmonella spp and Shigella spp) and of an ESBL in case of a group II Enterobacterales

(Enterobacter spp, Citrobacter freundii, Morganella morganiii, Providencia stuarti, Serratia spp, Hafnia alvei), were compared with reference methods and categorical agreement (CA), quantification and classification of errors determined. The overall CA for the Gram-negative panel was 97.7% for EUCAST and 96.9% for CLSI. For the Gram-positive panel, it was 96.3% for EUCAST and 96.4% for CLSI.

This methodology represents an alternative for direct ultra-rapid AST of Gram-negative and Gram-positive bacteria in bloodstream infections showing time-to-result <2 hours versus nearly 2 days with current methodology.

COLISTIN ACTIVITY EVALUATION THROUGH FLOW CYTOMETRY

Daniela Fonseca e Silva Porto, Portugal

The increasing prevalence of multidrug-resistant Gram-negative bacteria worldwide has led to a re-evaluation of the previously discarded antibiotic such as colistin. Despite its important role as last line therapy for otherwise untreatable infections, dosage guidelines for the use of colistin are not well defined and have led to treatment failure and increased colistin resistance.

For this reason accurate assessment of colistin susceptibility is crucial in multidrug-resistant Gram-negative bacteria and for the decrease of colistin resistance. Both EUCAST and CLSI recommend broth microdilution (BMD) to determine colistin susceptibility, however it is cumbersome and growth-dependent. In this presentation, a rapid and accurate assay by flow cytometry method (FASTinov®) for colistin susceptibility directly from colonies and positive blood cultures (BCs) is described with a turnaround time of 2 h versus 48 h required for BMD. This method represents an accurate alternative to standard BMD.

PARALLEL SESSION | ROOM 1

Hematology 1: Advances in diagnosis (Part 2)

Chair: **Paulo Lúcio** Lisbon, Portugal; **Maria José Oliveira** Porto, Portugal

ACUTE MYELOID LEUKEMIA WITH RECURRENT IMMUNOPHENOTYPIC ABNORMALITIES

Sergio Matarraz Salamanca, Spain

Acute myeloid leukemia (AML) is an exceedingly heterogeneous clonal myeloid disorder arising from stepwise accumulation of genetic events on hematopoietic stem, progenitor and/or precursor cells, leading to an oligoclonal expansion of leukemic cells in the bone marrow. The genetic background of AML involves a wide range of driver and passenger mutations, which impacts on patient risk stratification and further therapy decisions. For more than 20 years, baseline flow cytometry studies have brought upfront important correlations among the immunophenotype and genotype of leukemic cells in AML.

In this session we review most relevant immunophenotypic patterns and surrogate markers for prediction of underlying genetics in AML at diagnosis. The acquired knowledge over years, together with current analytical strategies being developed by EuroFlow for automated prediction of clinically relevant genetic alterations on AML leukemic cells, will conceivably help early risk stratification and therapy selection of these patients in clinical routine.

PROBING THE BONE MARROW MICROENVIRONMENT IN LEUKEMIA

Delfim Duarte Porto, Portugal

Hematopoietic stem cells (HSCs) are maintained by local bone marrow niches or microenvironments, including endothelial and mesenchymal stem cells. Leukemias, and in particular acute myeloid leukemia (AML), rely on cell extrinsic signals of the microenvironment to expand and evade chemotherapy. It has been shown that AML also remodels the bone marrow niche and indirectly affects non-malignant hematopoiesis. Flow cytometry is a key tool to study the hematopoietic system. When combined with other techniques such as imaging and RNA-sequencing, it allows a comprehensive study of the bone marrow microenvironment. I will discuss applications of flow cytometry to study the niche, with a focus on mouse models and our work on AML and vascular microenvironments.

ROOM 1

GECLID MEETING

Carmen Mártin Salamanca, Spain

This is the 10th year of GECLID quality assurance program, initiated in 2011, that started to run under the shared auspices of the Iberian Society for Cytometry and the Spanish Society for Immunology by mid-2012. The program is ISO 9001: 2015 certified for Interlaboratory comparisons in Diagnostic Immunology Tests for External Quality Assurance, with participants in Europe, South America and Australia. It comprises cytometry interlaboratory comparisons for lymphocytes, stem cells, innate and lymphocyte function, hematological malignancies, and rituximab follow up. In 2021, PD1 and T-cell repertoire schemes are available for the first time.

Along 2020, 82 cytometry labs took GECLID schemes, accounting for almost 20% of the total activity of GECLID (https://www.geclid.es/), which runs as well autoimmunity, histocompatibility and immunochemistry schemes.

Two main challenges are now our main aim: shortening of evaluation times, and increasing the number and repertoire of pathological samples sent. 2020 was a hard year for everyone and delays were assumed in order to include the maximum number of results from every lab. Recruiting cases was as well specially complicated due to the exceptional situation.

We are grateful to everyone participating, to our assessors within the Steering Committee, to those recruiting patients and sending samples. We hope we can be useful for at least 10 more years and we are ready to go on helping participant labs to our best.

16TH JUNE

CONGRESS

SIC Working Groups

SARS-COV2 and flow cytometry

Immunology 1: Immune monitoring

Flow cytometry in animal and oceanic sciences

Hematology 2: Measurable residual disease evaluation by flow cytometry: Advances and new perspectives

Flow cytometry in biotechnology

ROOM 1

SIC Working Groups

RECOMMENDATIONS FOR PNH SCREENING BY FLOW CYTOMETRY. RESULTS OF THE SIC PNH GROUP

Martin Perez Andrés Salamanca, Spain

Written summary not available.

UPDATE OF THE SIC WORKING GROUPS FOR HEMATOLOGICAL MALIGNANCIES

Martin Perez Andrés Salamanca, Spain; **Alba Torres Valle** Salamanca, Spain

Written summary not available.

PLENARY SESSION | ROOM 1

SARS-COV2 and flow cytometry

Chair: **Jordi Petriz** Barcelona, Spain; **Andrea Cossarizza** Modena, Italy

FLOW CYTOMETRY MONITORING OF SARS-COV-2 INFECTION REVEALS IMMUNE PROFILES AND KINETICS ASSOCIATED WITH DISEASE SEVERITY

Julia Almeida Salamanca, Spain

Coronavirus disease 2019 (COVID-19), caused by a novel coronavirus not encountered before by humans (named severe acute respiratory syndrome coronavirus 2, SARS-CoV-2), has raged for more than one year now, as declared by the WHO (World Health Organization). Clinical manifestations of COVID-19 patients range from mild disease (e.g. only fever or cough, or even asymptomatic disease, which we know now that occurs in most cases ->80%-) to critically ill cases with acute respiratory distress syndrome and septic shock. The wide spectrum of clinical expression of SARS-CoV-2 illness suggests that individual immune responses to SARS-CoV-2 play a crucial role in determining the clinical course after first infection, as the virus pathogenesis depends on the interaction between host immune genetic factors, environmental and virus agents. Therefore, understanding the underlying immune response would contribute to better know the ability of the different components of immune system in controlling the early phases of the infection, and hence to explain successful responses to SARS-CoV-2 infection or, on the other hand, to elucidate immune dysregulated and excessive inflammation mechanisms responsible for acute respiratory distress syndrome in severe/critically ill patients, as well as the components involved in long-term protective responses. Further, this knowledge becomes crucial to have prognostic/predictive biomarkers early in the course of disease, to promptly make appropriate decisions for improving clinical management. An extensive number of reports -mainly restricted to patients with moderate to severe/very severe disease- have actually described altered blood circulating immune cells in COVID-19 patients, which typically includes early lymphopenia at the expense of all the major lymphocyte subsets, together with an exacerbated plasma cell response, while more heterogeneous changes have been reported for innate cells. Importantly, the adaptive immune response has been shown to be critical in the later stages of COVID-19 infection, through generation of increased levels of neutralizing immunoglobulins against RBD (receptor-binding domain) and other domains of the spike viral protein. Although many large studies have described the immune profiles in COVID-19 patients, and related them with disease outcome, immune response dynamics during the course of SARS-CoV-2 infection and its possible correlation with clinical trajectory remain relatively unknown, and further, many of these studies have the following limitations: i) comparison of patient data with healthy donors has not been normalized according to the age of the controls; ii) long-term paired longitudinal studies have not been usually performed; iii) kinetics of immunological parameters has not been relativized to a common onset, such as the date of first COVID-19 symptom.

In order to solve these limitations and aiming at better understanding the basis of the immune response in COVID-19, as well as the precise association between certain immune profiles and the severity of the disease, we performed at the University of Salamanca and Hospital Universitario de Salamanca/IBSAL a global longitudinal analysis of innate and (cellular and humoral) adaptive immunity in COVID-19 patients (n=639 peripheral samples from 324 cases, from April 2020 to February 2021, from which a total of 136 samples from 23 samples were closely -every 24-48h- immune-monitored during the first 20 days since the onset of symptoms) selected across the spectrum of disease severity, ranging from asymptomatic/mild individuals to moderate, severe, and critically ill COVID-19 infected patients.

As regards the immune-cell kinetics during COVID-19 infection, our results show a systematic (early) decrease in dendritic cell counts followed by neutrophilia (with or without monocytosis), eosinopenia, basopenia and lymphopenia. Among lymphocytes, cytotoxic T cells decreased first, followed by CD4+ T cells, and subsequently, plasma cells (all isotypes and subclasses) peaked in blood (between day 6 and 14). As regards humoral response, we showed mild (transient) increased in IgM-specific antibodies, together with markedly increased and more stable amounts of IgG (until day +100) whereas IgA antibodies showed an intermediate profile (high but transient plasma levels). In general, between days 15 and 21 since the onset of symptoms, blood cell counts returned to normal values. Importantly, different immune profiles related to distinct severity outcomes of the disease. Accordingly, more severe cases were associated with higher counts of neutrophils and more pronounced eosinopenia and lymphopenia, together with higher (but delayed vs moderate/mild cases) counts of plasma cells and higher plasma levels of specific IgG and IgA against SARS-CoV-2, which also persisted for longer periods of time vs mild patients.

Overall, our analyses provide a comprehensive understanding of the diverse blood immune-cell kinetics during COVID-19 infection and show distinct immune-cell profiles in blood associated with the severity of the disease.

PARALLEL SESSION | ROOM 1

Immunology 1: Immune monitoring

Chair: **Artur Paiva** Coimbra, Portugal; **Maria José Oliveira** Porto, Portugal

NEW STRATEGIES FOR B-CELLS MONITORING IN INFECTION, AUTOIMMUNITY AND IMMUNODEFICIENCIES

Martin Perez Andrés Salamanca, Spain

The participants will learn which are the most informative phenotypic markers for subsetting the human B-cell subsets according to their maturation subset and the IgH subclass expressed.

After that, they will learn the reference values for each B-cell subset according to the age of the donor.

This information will be used to review which are the most significant alterations of these B-cell subsets in individuals suffering from infections, autoimmune diseases, and primary immunodeficiencies, and the clinical impact of these alterations.

SINGLE CELL APPROACHES FOR T CELL MONITORING IN VIRAL INFECTIONS AND CANCER

Sara De Biasi Modela, Italy

Over the past two decades, a pressing need to deeply profile cells responsible for the immune response has led investigators to integrate data obtained from traditional approaches with those obtained with new, more sophisticated, single-cell technologies, including high parameter flow cytometry, single-cell sequencing and high-resolution imaging. The introduction and use of these technologies have a prominent impact in the field of viral infection and cancer immunotherapy, allowing delving deeper into the molecular and cellular crosstalk between immune system cells, and fostering the identification of predictive biomarkers of response. In this talk, we will discuss how cutting-edge single-cell approaches are helping to point out the heterogeneity of immune cells in blood.

IMMUNOPHENOTYPE OF BLOOD MONOCYTE AND DENDRITIC CELL SUBSETS

Daniela Damasceno Salamanca, Spain

The importance of studying blood monocytes and dendritic cells resides in the need for the detection of a homeostasis imbalance which occurs each time we are exposed to external agents, which enter our organism and induce an innate immune response. For the detection of these alterations is crucial to have consistent criteria for the identification of the main innate cell subsets and their minor subpopulations as well as extensive data on healthy donors in order to compare it with data from patients.

To accomplish these goals, we have developed a combination of the most informative markers in the scope of the Euroflow-Periscope consortium, allowing for the identification and classification of monocyte and dendritic cell subsets. Among the markers described, IgE high-affinity receptor and Slan molecules are reviewed, as they were recently found to be expressed in a subset of classical monocytes/dendritic cells and in a subset of non-classical monocytes, respectively. Moreover, myeloid derived suppressor cells, CD100+ pre-dendritic cells, hematopoietic progenitors and other immune cells are also identified.

Finally, we show different examples for monitoring these cell compartments both in healthy individuals and in other clinical settings, such as the monitoring of tissue damage in total hip arthroplasty, coronary artery disease, and monoclonal gammopathies.

PARALLEL SESSION | ROOM 2

Flow cytometry in animal and oceanic sciences

Chair: **Vitor Vasconcelos** Porto, Portugal; **Ana Faustino** Évora, Portugal; **José Carlos Segovia** Madrid, Spain

LOW CYTOMETRY IN VETERINARY ONCOLOGY

Fulvio Riondato Turin, Italy

Currently the most important diagnostic application of flow cytometry in veterinary oncology is for the characterization of lymphomas in pets. Flow cytometric analysis helps in the differentiation between neoplastic and reactive nodal enlargement; the main features considered are cell size (FSC), the prevalence of a population with a unique immunophenotype vs a mixed population, the presence of aberrant patterns. Immunophenotyping is fundamental for the correct classification of lymphomas according both to the histologic and cytologic classifications (WHO and Kiel updated, respectively) where the recognition of the cell lineage (B vs T) is mandatory. In the case of T-zone lymphomas flow cytometry is predictive of the WHO subtype (CD45-negative T-cells).

Additional diagnostic information are obtained through the determination of the proliferative activity (Ki67 and S-phase fraction), useful for the discrimination between low grade/indolent and high grade/ forms. Flow cytometry is commonly used for staging the disease looking for peripheral blood and bone marrow infiltration; the analysis of splenic and hepatic US-guided aspirates can also be easily performed. It is also run to assess MRD at the end of chemotherapy.

Different flow cytometric data with prognostic significance have been described: Ki67% in large B cell lymphomas (LBCL), MHC-II expression in B-cell lymphomas, percentage of peripheral blood infiltration in MZL and bone marrow infiltration in LBCL and MZL, nodal MRD in DLBCL treated with chemo-immunotherapy, nodal non-neoplastic lymphoid populations at the time of diagnosis in DLBCL.

Recently we developed a flow cytometric approach to characterize canine mast cell tumor and to detect the presence of mast cells in lymph nodes and other organs. The clinical significance of flow cytometric data compared to the reference classification system remains to be determined. Finally, the routine panel used for the analysis of effusions in dogs can be implemented with the evaluation of cytokeratin, vimentin and desmin in the non-hemopoietic population, thus providing useful indication for the differential diagnosis of mesothelioma and carcinoma.

ISOLATION AND IMMUNOPHENOTYPING OF PERIPHERAL BLOOD AND INTRAEPITHELIAL AND LAMINA PROPRIA

Beatriz Agulla Pérez Madrid, Spain

Inflammatory bowel disease (IBD) is an unknown etiology entity characterized by the persistence and recurrence of gastrointestinal clinical signs. It is considered the principal cause of chronic vomiting and diarrhea in the dog. Interrelated immunological, dietary, genetic and environmental factors appear to play a role in the pathogenesis of this immune-mediated disease. Its diagnosis is obtained after carrying out an exclusion protocol of all underlying causes for intestinal inflammation accompanied by histological evidence of mucosal inflammatory infiltrate.

In human medicine, the great advances in IBD immunopathogenesis have allowed the development of new therapeutics based on immunotherapy and the description of useful immune biomarkers. However, the knowledge of immune system alterations during IBD course in the canine species is limited. For this reason, in the present study the peripheral blood and the intestinal immunophenotype of dogs with IBD were assessed by flow cytometry.

In order to employ flow cytometry for intestinal immunophenotyping, firstly a protocol for intraepithelial and lamina propria duodenal lymphocytes (IEL and LPL, respectively) isolation were described because for LPL this technique had not been previously reported in the canine species. In this regard, a protocol for each intestinal location, which provides the necessary quality criteria for flow cytometry evaluation of these lymphocytes in terms of viability, purity and cell yield, was selected.

Then, for the evaluation of the potential immunophenotype alterations in dogs with IBD, it was first necessary to characterize the lymphocyte populations that seem to have relevance in this disease by flow cytometry (considering the lymphocytes subsets T, Th, Tc, double positive T cells, double negative T cells, activated Th and Tc, IFN-γ and IL-4 producing T in both peripheral blood and intestinal biopsie) in 16 healthy dogs of different age, sex and breed. These data allow us to have our own reference ranges. In addition, the comparative study of these lymphocytes distribution in the three immune compartments analyzed: peripheral blood, epithelium and the duodenal lamina propria, showed great differences in the composition of these cell types. This fact support the importance of its independent study. Furthermore, a greater similarity has been found between the blood and the duodenal lamina propria compartments.

The knowledge of physiological values for normal canine blood lymphocytes and IEL and LPL enabled to assess the potential alterations developed in dogs with IBD (in 36 animals). The dogs with IBD showed an increase in the percentage of activated Th and in the percentage and in the relative count of the Treg and Th2 from bloodstream than healthy dogs. In the intestine, a decrease in the epithelial percentage of Th, double positive T cells, activated Th and in the CD4/CD8 ratio; and an increase in the epithelial percentage of Tc and producing IFN-γ lymphocytes and lamina propria Treg lymphocytes percentages was observed in the disease dogs. The findings show the alteration of both systemic and intestinal immunophenotype in the canine IBD course.

MARINE VIRAL ECOLOGY THROUGH THE BEAM OF FLOW CYTOMETRY

Gonçalo Piedade Texel, Netherlands

Microbes are the base of the marine food web, making up >70% of the living biomass in the seas and oceans. The ecology and evolution of viruses (10^30 in the global oceans) are entwined with that of their hosts, making them key modulators of microbial populations. The high-throughput characteristics of flow cytometry (FCM) enable an unprecedented detailed investigation of the ecological impact of viruses in the marine environment. Here I provide some examples; firstly, the combination with FCM promotes high-resolution field measurements for virus and bacteria abundances in the Southern Ocean. Furthermore, it allows for estimations of viral lysis rates of bacteria. The study exemplifies that high viral lysis rates drove lower bacterial abundances under constant bacterial production for the Weddell Gyre region. FCM also permits a more directed study of viral lysis and grazing rates of phytoplankton.

Our results show that viral lysis is an important mortality factor for all the phytoplankton populations discriminated by flow cytometry during summer in the Northeast Atlantic Ocean. It revealed a switch from grazing to viral lysis with decreasing latitude, with important implications for the biogeochemical cycles. Likewise, my research using an Arctic phytoplankton host-virus model system benefit greatly from FCM, allowing a detailed analysis of how environmental factors affect virus infectivity and proliferation. Low light availability was found to constraint while high temperature promoted virus production with important implications for the Arctic marine ecosystem. Results will be discussed in relation to natural dynamics and global warming expectations. Currently, we connect our FCM-based Antarctic viral ecology research with viral and host metagenomics diversity studies.

HOW AUTOMATED FLOW CYTOMETRY CAN IMPROVE OUR UNDERSTANDING OF THE PLANCTON STRUCTURE, DISTRIBUTION AND FUNCTIONING

Gérald Grégori Marseille, France

Flow cytometry has greatly contributed to improve our knowledge of aquatic trophic food webs through the exploration at the single-cell level of the various planktonic communities such as phytoplankton, bacteria, marine viruses. Apart from counting these planktonic particles the analysis of variations in autofluorescence, light scatter and the use of fluorescent stains has demonstrated its power in elucidating the physiology and adaptations of organisms to the natural environment both in natural samples and in culture. Automated in situ flow cytometry makes it possible to sample the Ocean with a high spatial and temporal resolution, offering a new opportunity to address complex questions and bring new answers to better understand the functioning of the marine ecosystem. For instance, model simulations and satellite observations have shown that ocean dynamics at fine scales (1–100 km in space, day–weeks in time) strongly influence the distribution of phytoplankton. This temporal scale is similar to that of many biological processes, such as phytoplankton growth, suggesting a physical and biological coupling. To better characterize this coupling, both physical and biological measurements in situ are mandatory. However, the observations of fine scales constitute a challenge due to the difficulties of sampling at high spatio-temporal frequency. In this presentation, we'll present how a satellite-based adaptative and Lagrangian strategy coupled with a high-resolution physical-biological sampling, using an automated flow cytometer installed onboard, has been performed to follow and describe fine-scale structures in the Mediterranean Sea.

PARALLEL SESSION | ROOM 1

Hematology 2: Measurable residual disease evaluation by flow cytometry: Advances and new perspectives

Chair: António Campos Porto, Portugal; **Maria Jorge Arroz** Lisbon, Portugal; **Fernanda Trigo** Porto, Portugal

CURRENT FLOW CYTOMETRIC APPROACHES TO MEASURABLE RESIDUAL DISEASE DETECTION ON HEMATOLOGIC MALIGNANCIES

Juan Flores Montero Salamanca, Spain

Minimal residual disease detection by flow cytometry currently holds a relevant place on treatment monitoring strategies of hematologic malignancies; although, over the years, this consideration has been recognized at different paces for different disease categories. The utility of MRD flow cytometry strategies to measure the direct effect of the therapeutic interventions, compare this effect among different therapeutic regimes, re-stratification of patient's risk and to anticipate prognostic information directly linked to classic clinical endpoints is now more broadly recognized. During the presentation, we will review the most recent and widely used flow cytometry approaches for MRD detection in onco-hematology and underline the characteristics of a successful strategy. In addition, the aspects currently active for innovation and the opportunities for improvement will be discussed.

MRD DETECTION: WHAT IS NEW IN AML AND MM

Bruno Paiva Pamplona, Spain

The landscape of acute myeloid leukemia (AML) and multiple myeloma (MM) have changed considerably in the past two decades regarding new treatments, insight into disease biology and innovation in the techniques available to assess measurable residual disease (MRD) as the most accurate method to evaluate treatment efficacy. The sensitivity and standardization achieved by some flow cytometry methods, together with unprecedented rates of complete remission (CR) induced by new regimens, raised enormous interest in MRD as a surrogate biomarker of patients' outcome and endpoint in clinical trials. By contrast, there is reluctance and general lack of consensus on how to use MRD outside clinical trials. Here, we discuss critical aspects related with the implementation of MRD in clinical practice.

PARALLEL SESSION | ROOM 2

Flow cytometry in biotechnology

Chair: **Rui Gardner** New York, USA; **Lillian Barros** Bragança, Portugal

APPLICATION OF FLOW CYTOMETRY IN THE ASSESSMENT OF NATURAL PRODUCTS BIOACTIVITIES

Rui Abreu Bragança, Portugal

The interest in natural products characterization and application in different research areas has increased sharply in the last decade. It is acknowledged that natural matrices have tremendous potential as sources of bioactive extracts and individual natural compounds to be used in a large variety of biotechnology-related research fields, including food, cosmetic and pharmaceutical applications. Many bioactivities are studied to assess the potential of natural products, including antioxidant, anti-inflammatory, antimicrobial, and antitumoral activities. Flow cytometry can be applied in the analysis of these bioactivities. In this presentation, the focus will be on the use of flow cytometry to study the antitumoral activity of several natural matrices, including different plants and mushrooms, by analyzing their effect on the onset of apoptosis and the impact on the cell cycle process. An example of the use of flow cytometry in the study of synthetic compounds will also be presented, as the principles of analyzing bioactivities of both natural and synthesized compounds are similar. Finally, we will demonstrate that flow cytometry is an instrumental methodology in discovering natural products' bioactivities and elucidating the respective cellular and molecular mechanism of action.

FLOW CYTOMETRY IN FOOD MICROBIOLOGY AS RELATED TO CHEESE PRODUCTION

Martin Wilkinson Limerick, Ireland

Cheese production involves the conversion of milk into a flavourful semi solid product using selected Lactic Acid Bacteria (LAB) as an integral part of the production and ripening processes. LAB are added to cheese milk as "starter" strains having been selected for consistent acid production, resistance to bacteriophage and production of typical flavours during ripening. On the day of manufacture in the cheese vat, LAB generate lactic acid to reduce the pH value from ~6.6 in milk to ~ 5.30 at salt addition stage. The second major contribution of LAB starter strains is from their proteolytic enzyme system which consist of external Cell Envelope Proteinase (CEP) and a range of intracellular peptidase enzymes including the Pep X peptidase responsible for de-bittering and assisting in generation of flavourful free amino acids. Ripening and flavour development of cheese is generally attributed to the action of LAB proteolytic enzymes which are released from non-viable, permeabilised and autolysed cells. The application of flow cytometry (FCM) to understanding the microbiology of cheese ripening has been considerable especially in our understanding of strain-related differences that exist in autolysis, permeabilization, intracellular release and how they impact upon proteolysis and cheese flavour. Initially, prior to the application and development of flow cytometry methods to study LAB in cheese, our understanding of LAB physiology was limited to data from viable plate counts and detection of activity of released marker enzymes such as Lactate Dehydrogenase (LDH) and Pep X. The use of flow cytometry with differential staining methods (SYTO9/PI) when combined with enzyme release and localisation studies has greatly advanced our insights into the influence of cheese production processes on the response of differing starter strains. We now know that the cooking temperature step of Cheddar cheese manufacture, where a shift from 32 to 38C occurs over 30 mins, has major strain related effects on the commencement of the permeabilization and autolysis process and indeed appears to greatly influence both intracellular enzyme release and accessibility. The importance of this cooking temperature step on LAB strains is reflected in differing rates of ripening and flavour development brought on by differing proteolysis levels from the intracellular enzymes released by the LAB strains. Hence, FCM when combined with enzymology and flavour development studies has allowed a greater optimisation of control of the ripening process. In terms of selection of starter LAB strains for particular cheese application, FCM has enabled the new criteria to be used to select LAB strains for use in the production of low salt cheese where starters can be selected for optimised enzyme release in cheeses with altered compositional environments.

SURVIVAL AND METABOLISM OF HYDROXYCINNAMIC ACIDS BY DEKKERA BRUXELLENSIS IN MONOVARIETAL WINES

José António Couto Porto, Portugal

Volatile phenols in wines are responsible for unpleasant aromas, which negatively affect the quality of the wine. These compounds are produced from the metabolism of hydroxycinnamic acids, mainly by the yeasts Brettanomyces/Dekkera. Relevant data, potentially useful to support decisions on how to manage the risk of contamination of wines by Brettanomyces/Dekkera, according to the grape varieties used in the vinification, is important to the wine industry.

Therefore, the aim of this work was to evaluate the survival and the metabolism of hydroxycinnamic acids by Dekkera bruxellensis in monovarietal wines. Yeast growth and survival were monitored in fifteen wines, five from each of the grape varieties Touriga Nacional, Cabernet Sauvignon and Syrah, inoculated with a strain of D. bruxellensis. Yeast culturable populations of 107 CFU mL-1 were reduced to undetectable numbers in 24 h in all wines. Plate counts of 104-106 CFU mL-1 were, however, detected after 48 h in most of Touriga Nacional and Cabernet Sauvignon wines and later in Syrah. Viability measurement by flow cytometry showed that a significant part of the populations was in a viable but non-culturable state (VBNC). The time required for the recovery of the culturable state was dependent on the wine, being longer on Syrah wines. Besides the production of ethylphenols, the metabolism of hydroxycinnamic acids by VBNC cells led to the accumulation of vinylphenols at relatively high levels, independently of the grape variety. The flow cytometry methodology showed a higher survival capacity of D. bruxellensis in Touriga Nacional wines, which corroborates with the higher amounts of volatile phenols found on this variety.

17TH JUNE

CONGRESS

Flow cytometry in solid tumors

New frontiers in FCM applications

Extracellular vesicles, progression and treatment resistance in cancer

Accreditation and certification

PARALLEL SESSION | ROOM 1

Flow cytometry in solid tumors

Chair: Júlio Oliveira Porto, Portugal; **Carmen Jerónimo** Porto, Portugal

PREPARE TO EXPLORE THE TUMOR MICROENVIRONMENT

Martijn Van Baalen Amsterdam, Netherlands

Biopsies from solid tumors are often small and rare samples. Since this material can only be used once, it's important to obtain a single cell suspension of high quality to probe.

In the quest to explore the immune component of the tumor micro environment, preparation and optimization of tissue dissociation are key. The most important aspects in the experimental design phase are covered to obtain high cell yield, viability, and retrieve high quality data from the cells of interest. This presentation has a focus on analysis of immune cells from solid tumors, but the provided information is also applicable to other cellular assays from a wide range of tissue samples.

Learning outcomes

› Learn why optimization is key when probing the immune component of solid tumors.
› Learn about key elements that can be optimized in the sample preparation phase
› Understand why flow cytometry experiments are hypothesis driven

THE POTENTIAL OF FLOW CYTOMETRY FOR IMMUNOSCORE ANALYSIS OF HUMAN LUNG TUMORS

Alexandre Corthay Oslo, Norway

Measuring the state of the antitumor immune response in order to provide each cancer patient with an "immunoscore" has a great potential to predict the risk of recurrence and the response to therapy. In this lecture, I will show how flow cytometry can be used for detailed analysis of tumor-infiltrating immune cells in human non-small cell lung cancer. The staining panels that we have established can in principle be used to investigate the immune cell composition of any type of human tumors. I will also discuss the advantages and limitations of using flow cytometry to generate an immunoscore in comparison with immunostaining of formalin-fixed, paraffin-embedded (FFPE) tumor sections.

ANALYSES OF THE PERIPHERAL IMMUNOME IN CANCER IMMUNOTHERAPY TRIALS

Renee Donahue Bethesda, USA

Dr. Donahue's talk on "Analyses of the Peripheral Immunome in Cancer Immunotherapy Trials" will provide evidence that analyses of the peripheral immunome, which is much more easily accessible than the tumor, can provide valuable information. Interrogating the peripheral immunome can provide information on the immune status of patients prior to therapy, which can help to identify those patients most likely to benefit from immunotherapy or combination therapies. In addition, evaluation of the peripheral immunome can provide mechanistic information on the effect of various therapies (including "non immune based therapies", immunotherapies, or combinations of therapies) on immune cell subsets that are not easily identified in biopsies. Finally, interrogating the peripheral immunome and identifying differences in responding and non-responding patients can help to inform rational combination approaches for future studies.

PARALLEL SESSION | ROOM 2

New frontiers in FCM applications

Chair: Alfonso Blanco Dublin, Ireland; **Paula Ludovico** Braga, Portugal

NEW FRONTIERS IN FCM APPLICATIONS

Paul Robinson Indiana, USA

The importance of rapid identification of viral structural sequence quickly followed by cellular subset and activation molecules identification to determine clinical impact was highlighted during the early days of the COVID-19 pandemic. Apart from the obvious importance of sequencing, one of the earliest clinical discoveries was the change in T-cell subsets and the dramatic impact of upregulation of cytokines causing cytokine storms with dramatic clinical impact on patient morbidity. These discoveries were made within weeks of the earliest stage of the pandemic and were made using flow cytometry – a single cell technology that has become a go-to technology in immunology. For many years flow cytometry has had a place of significant importance to a few very specific subsets of scientists, but the COVID pandemic most definitely enhanced the importance of the technology.

However, technologies are not stable entities. They change and adapt to changing demands. If they don't, they become redundant and end up as footnotes in history! Fortunately, for many of us, flow cytometry has adapted – not as quickly as Covid perhaps, but sufficient to survive for perhaps another 20 years as a highly relevant technology. One example of this morphing is the transition from polychromatic to spectral cytometry. I gave my first talk on this in 2004 when I was convinced spectral flow cytometry would become the only approach within a few years. Of course I was wrong, but only in the timing, although some would argue that timing is everything!! My sense of the present pace in spectral instrument development is that that 5 years may well be accurate this time! I guess we will see if my predicative ability has improved!

But there is more than just spectral cytometry on the horizon. There is a potential for a 2nd generation spectral technology that we have been working on that may provide many more features that we currently consider when we design our experiments. The presentation will discuss the engineering developments in next-generation of technology that will open up new frontiers in biotechnology research and most importantly in both research and clinical diagnostics. The presentation will outline new sensor technology and how this has generated huge datasets that require advanced analytical toolsets for automated diagnostics.

FLOW KARYOTYPING AND CHROMOSOME SORTING

Òscar Fornas Barcelona, Spain

Flow karyotyping was developed at 70´s to analyze human chromosomes. The strategy was based on their relative DNA content and base pair composition with a double DNA staining, using Hoechst 33258 and chromomycin A3 to stain AT base pairs and CG base pairs respectively. During its evolution, some modifications were included to improve its resolution. Chromosome sorting has been used to study variations between human chromosomes, to detect chromosomal abnormalities, to map genes and to generate chromosome-specific libraries. This cytogenetic application can also be used to detect structural DNA variants such as large genomic deletions, translocations and copy-number variation on specific chromosomes when combined with FISH. However, two decades of whole genome sequencing and its amazing improvement of genomics field, has relegated it to as second division application. We have recovered bivariate flow karyotyping from the past to combine it with subsequent DNA sequencing, without amplification, by using the new MinION device from Oxford Nanopore sequencing technology as a new strategy to facilitate the assembly of complicated genomic territory such as human Y-chromosome. We propose this workflow as a potential solution to assemble structurally complex chromosomes, or the study of very large plant or animal genomes that might challenge traditional assembly strategies.

FLOWFISH: ASSESSMENT OF RELATIVE TELOMERE LENGTH BY FLOW CYTOMETRY

Cláudia Nóbrega Braga, Portugal

Telomeres are DNA sequence repeats that cap chromosomes' ends, providing protection and stabilization to genetic material. As cells go through somatic division, telomeres get shorter. With aging, an increased proportion of cells that went through several cycles of division and consequent telomere shortage accumulate and for this reason telomeres' length become good surrogate for both chronological and biological aging.

By coupling the usage of a fluorescent probe complementary to the telomere DNA sequence repeats with fluorescent antibodies, Flow FISH allows a high throughput analysis of the average telomere length of different cell subsets on heterogeneous cell suspensions. In this presentation, I will perform an overview on the implementation of the Flow FISH protocol in our lab, followed by our latest results regarding the evaluation of blood T cells relative telomere length in the context of multiple sclerosis.

CYTOMICS IN IMMUNOTHERAPY TREATMENT DECISION-MAKING

Jordi Petriz Barcelona, Spain

Approximately 1.75 million patients globally suffer from blood cancer and many are in need of new treatment options. Among them, multiple myeloma (MM) is the second most common hematologic cancer, comprising 10–15% of all hematopoietic malignancies and causing 20% of all deaths from these diseases. MM is characterized by the accumulation of malignant plasma cells in the bone marrow (BM), constituting a critical microenvironment for the survival, expansion and chemoresistance of myeloma cells. Although new therapies have markedly improved the results in the treatment of MM, today remains incurable, but manageable once diagnosed.

Recent studies have shown that MM tumor cells play a critical role in resistance to chemotherapy, immunotherapy and radiotherapy, as they are significantly less refractory to these therapies than other cells. In addition, the presence of Myeloid-Derived Suppressor Cells (MDSC) in the microenvironment of BM in early stage MM contributes to local suppression of the immune responses through the PD-L1 / PD-1 pathway. MDSCs constitute a heterogeneous population that expands during cancer, inflammation and infection, playing a critical role in the progression of MM, being considered a therapeutic target for this disease.

The recent encouraging results from antibodies targeting programmed cell death protein 1 (PD-1) and B7 homolog 1 (B7H1; also known as PDL-1) for the treatment of various advanced human cancers show that immunomodulatory therapy has come of age. MEDI4736 is a human monoclonal antibody directed against PD-L1, which helps tumors avoid detection by the immune system. Tumor cells use PD-L1 to turn off the immune system just as it begins to mount a response against them. MEDI4736 helps turn the immune system back on, allowing it to continue its attack on cancer. Thalidomide, an immunomodulatory drug (IMiD), has improved the response rate and survival of patients with MM. Moreover, lenalidomide and pomalidomide, the second generation of IMiDs, have emonstrated more potent anti-MM, anti-inflammatory and immunomodulatory activities than thalidomide. Although these new therapeutic approaches have improved MM clinical outcomes, most MM patients show drug resistance and eventually relapse. The aim of this study was to develop an in-house cytomic assay that can be performed on PB/BM diagnostic samples. The assay can be used as a guide to predict response to anti PD-L1 directed therapies. This new experimental approach may be useful to improve existing immunohistochemical methods and more follow-up, as a vital part of patient safety.

PARALLEL SESSION | ROOM 1

Extracellular vesicles, progression and treatment resistance in cancer

Chair: **Lúcio Lara Santos** Porto, Portugal; **Helena Vasconcelos** Porto, Portugal

EMPLOYING FLOW CYTOMETRY TO EXTRACELLULAR VESICLES POPULATION ANALYSIS

Bruno Costa Silva Lisbon, Portugal

Extracellular Vesicles (EVs), membrane vesicles released by all cells, are emerging mediators of cell-cell communication. By carrying biomolecules from tissues to biofluids, EVs have attracted attention as non-invasive sources of clinical biomarkers in liquid biopsies. EVs-based liquid biopsies usually require EVs isolation before content analysis, which frequently increases sample volume requirements. We here present a Flow Cytometry (FC) strategy that does not require isolation or concentration of EVs prior to staining. By doing so, it enables population analysis of EVs in samples characterized by challenging small volumes, while reducing overall sample processing time. To illustrate its application, we performed longitudinal non-lethal population analysis of EVs in mouse plasma and in single-animal collections of murine vitreous humor. We also utilized it to monitor tumor-associated EVs populations in metastatic pancreatic cancer patients.

UNDERSTANDING CELL–NANOPARTICLE INTERACTION: A NEW GATEWAY TO DISEASE THERAPEUTICS

Sofia Antunes Costa Lima Porto, Portugal

Nanoparticles and their interaction with human cells have been a focus of many groups during the past decade. The progress in the field of understanding and harnessing the interactions of nanoparticles with different cell types will be present and discuss. Nanotechnology and the hereby produced nanomaterials have promised to make use of specific properties of supramolecular assemblies and nanomaterials so that hitherto inaccessible effects can be exploited for new applications.

In biology and health, superparamagnetic iron oxide nanoparticles have been used for cell selection and as magnetic resonance imaging (MRI) contrast agents. Furthermore, uptake of nanoparticles into a wide variety of cells is an effect that seems to be specific for materials in the range of 50−200 nm. Surface modifications (positively or negatively charged side groups of the polymers, amino acids, or peptides/proteins) enhance this uptake. Knowledge about factors influencing cellular uptake, like size, surface properties, cell type, and endocytic pathways, enables optimization of labelling and selection of cells and nanoparticles for applications in vitro and in vivo. Here will focus on how nanoparticles can cross the biological barriers, namely gastrointestinal and skin using flow cytometric tools.

PARALLEL SESSION | ROOM 2

Accreditation and certification

Chair: **Juana Ciudad** Salamanca, Spain; **Tiago Guimarães** Porto, Portugal

KEY PERFORMANCE INDICATORS IN FLOW CYTOMETRY ASSAYS

Juana Ciudad Salamanca, Spain

In the present context, an indicator is a parameter or item that we can use to know or monitor the characteristics, the intensity, or magnitudes of an event at a concrete moment or to predict their evolution in the future.

In a Quality Control System, indicators are a very interesting tool to control the quality and the ongoing of the process. They must be part of a system very well designed to be capable of measuring the fulfilment of the established technical characteristics for each process.

Process by process, we can define various indicators, but the number is not the most important thing. More than having a high number of indicators, it is preferable to have less indicators, but assure that the ones selected are capable of obtaining all the critical information we need to take decisions, without spending too much time and resources.

Moreover, each indicator shall be focused in the decision making process, and designed to assure it Is easy to measure, easy to understand and easy to be introduced in the day-to-day work, being also logical and, if possible, capable of generating standards.

The information that the indicators produce must be revised every 3, 6 or 12 months, depending on how critical the indicator is. With the results of this analysis, we shall then establish the values or the cut offs to take decisions and the decisions shall consider the different situations. Of course, for the cut offs to be useful, they must also be easy to understand and visualize.

Considering standards, ISO 9001 only says that laboratories must establish what they need to measure, how and when this assessment shall be done and the need to analyse the results. Standards do not specify indicators but these are the best way to do this analysis, obviously. ISO 15189 establishes the need to have indicators to monitor the ongoing of all processes in the preanalytical, analytical and postanalytical phases, making a planification with objectives, methodology, interpretation, cut offs or limits to make decisions and to do a planification of what shall be done in all situations.

Although each Quality System needs to look for the best indicators, I would like to review some of them, the most essential in general, because a review of all indicators is impossible. So, I will try to resalt some ideas to look for indicators with some examples that I consider we do not forget in any cases.

First, we want to control Uncertainty. The evaluation of the uncertainty will be done making the control of all the implicated process (because in flow cytometry other ways are not possible): samples, reagents, instruments/ equipment, process of preparing, acquiring and analysing tubes.

How can we do that?

This can be evaluated through the information of the management of Non-conformities. The management of the non-conformities, if these are well designed, evaluated, and revised overtime, is a very good tool and indicator of Quality.

Obviously, to cover all the process, we need to analyse Non-conformities related to:

1. Test request, samples, and registration: mainly, the rates of rejected samples, but also incidences related with registration or other. In fact, the rate of samples processed with serious incidences is one of the best indicators in clinical assays because high rate of rejected samples is indicative that the chain of communication between the users/customers and the laboratories is failing. Again, this is only possible if these incidences/non-conformities are properly registered.
2. Problems with reagents in all the chain of their use: from shopping to use in routine, including checking, not well performing antibody staining, and others, including stock control.
3. Rate of errors in technical processes (antibody staining, acquiring tubes in the flow cytometers....) and other processes, like rates of errors in the elaboration of reports.
4. Time of response: Non-conformities related to the delivery of results and reports. For this, laboratories should fix the optimum turnaround times for results and reports in different assays. Respecting these times is recommended, as long as they are reasonable, acceptable, based on the characteristics of each assay or in the times used by similar laboratories or centres.
5. Record also incidences in the Cytometer, that may be related to daily checking with control beads, to out of service errors and other technical interventions in the equipment, like preventive maintenance operations.
6. Results obtained in the performance of Internal and External Quality Control should be exhaustively analysed as well, to detect problems and to focus the laboratory on obtaining better results along time.
7. Record information in non-analytical processes: also measure the compliance of critical points in non-analytical processes, like personnel/staff, documents and their management, etc.
8. Customers and users' satisfaction. Customers and users' satisfaction are important but it is not enough to address these with satisfaction surveys. It can be important to also analyse requests, claims and other interactions that obviously must be registered too, in a proper and thoroughly manner, even if they were made by telephone.

Conclusion

Very simple: not too many indicators, but very focused ones are necessary instead, to make changes and decisions, and to get better information, based in properly registered data. In this approach I would consider the following as essential: percentage of rejected samples, rate of errors in registration, technical processes, reports, among others.

REVISITING RISK MANAGEMENT IN THE LIGHT OF THE COVID-19 PANDEMIC: STRATEGIES AND TOOLS TO IMPROVE SAFETY AND QUALITY IN FLOW CYTOMETRY LABORATORIES

Catarina Martins Lisbon, Portugal

Risk assessment is an important exercise, mentioned in international standards, quality schemes, and biosafety guidelines. However, instead of being a combination of inputs from researchers, laboratory staff, and health and safety committees, this exercise is often too clerical and may became segregated from daily laboratory routines.

The explosion of the COVID-19 pandemic brought risk analysis and risk assessment into the spotlight. For many clinical and research centers, COVID-19 exposed the need to deepen risk assessment practices in routine laboratory work, as laboratories (including flow cytometry ones) were forced to adapt to this new challenge. In fact, risk assessment, aimed to be a dynamic resource, merging contributions from all involved parts of the process, is vital to cope with the continuous evolution of biomedical sciences and technologies. By adjusting guidelines and procedures according to the risks identified, it may notoriously improve laboratory practices and safety.

However, risk assessment is not restricted to biosafety applications, and it is also a relevant tool for improving the quality of services to patients and clients in clinical and research settings. Indeed, the analysis of more relevant processes, planification activities or the implementation of changes in laboratory routines should always consider the risks and potential failures associated, to improve the efficiency and the quality of the methods and practices implemented. As many standards state, improvement should be directed at priority areas based on risk assessments.

Hence, it is crucial to understand the centrality of risk assessment practices for the development of safety and quality procedures in flow cytometry laboratories. Thus, with this talk, we aim the explore the concepts of risk analysis, risk evaluation and risk control, and their application in a continual improvement philosophy. For that purpose, different

strategies, and tools applicable to risk assessment shall be addressed, including ISO 22367 and FMEA (Failure Mode and Effect Analysis), looking into their usage in flow cytometry laboratories.

HOW TO ADAPT AND RISE TO A PANDEMIC

Lola Martinez Madrid, Spain

A core technology platform is by nature a multi-user environment where different members of the research community interact. As part of the work in a core lab users with different levels of experience come to get trained by the core staff. It is typical of the core lab to count with biosafety plans to ensure the work is carried out fulfilling biosafety standards and regulations but those plans are focus on the samples and reagents and how to manage them within the lab and what is the correct protocol to dispose of such samples according to their biological classification.

Early last year the Covid19 pandemic sent us all home as a lockdown was put in place into many countries worldwide and many core labs either closed down or drastically reduced their workload. To kept staff and users engaged many institutions developed online workshops, which were quite useful at the time and continue being good tools for our users. It was also the time to re-shape our biosafety plans to include the risk assessment of our own users as SARS-CoV-2 is an airborne transmitted virus as well as to change our cleaning and booking policies.

Upon the end of lockdown in early May we all start rolling back into work and core lab need it to reconfigure their spaces to cope with the new social distancing rules as well as kept with the staffing availability and room occupancy levels. Core instruments were relocated when possible to allow for their use complying with the recommendations from the health authorities on social distancing - usually core labs design follow many instruments on the same space which wasn't the most practical approach under these new normality rules.

Another main point that core labs have to change their training activities to move them into the virtual world for which after the initial struggles in transitioning face-to-face into virtual training for which remote access tools and pre-recorded educational and training materials have prove a unique opportunity to advance their training program."

BIOSAFETY GUIDELINES FOR THE MANAGEMENT OF POTENTIALLY BIOHAZARDOUS SAMPLES - SARS COV2

Óscar González Salamanca, Spain

The COVID-19 pandemic has showed that most of our laboratories weren´t prepared for working with samples suspicious of containing an airborne dangerous virus.

In this presentation we will review some aspects to consider when we must adapt our laboratories to this new situation and the recommendations to control the risk involved in the handling of SARS-CoV2 samples or suspicious of being contaminated with this incredibly spreadable virus.

We will go over all the hierarchy of risk controls, from the design of the laboratory, reviewing the characteristics of BSL2 and BSL3 labs, to the PPE that we should wear when it is impossible to eliminate completely the risks, taking special attention in the measures that we should take to reduce or eliminate the generation of aerosols.

18TH JUNE

CONGRESS

CAR-T cells therapy and flow cytometry application

Immunology 2: Immunotherapy

Light beyond the tunnel: Cytometry after horizon 2020

PLENARY SESSION | ROOM 1

CAR-T cells therapy and flow cytometry application

Chair: **Alberto Órfão** Salamanca, Spain; **José Mário Mariz** Porto, Portugal

ADOPTIVE CELLULAR IMMUNOTHERAPIES DRIVEN BY CHIMERIC ANTIGEN RECEPTORS

Pablo Menéndez Barcelona, Spain

In this sesion, we will review the concept of cancer immunotherapy with special focus on the main T-cell redirecting strategies. We will then discuss the available biological and clinical data on state-of-the-art CAR T-cell approaches for B-cell malignancies including B-cell ALL, lymphomas and multiple myeloma, as well as T-cell malignancies, and acute myeloid leukemia. We will finally discuss current biological barriers to advance CAR T-cell therapies in solid tumors, and will finally propose the main research and innovation areas expected to advance the field over the next few years.

PLENARY SESSION | ROOM 1

Immunology 2: Immunotherapy

Chair: **Aberto Órfão** Salamanca, Spain; **Artur Paiva** Coimbra, Portugal

MONITORING CAR-T CELLS IN LYMPHOID MALIGNANCIES BY FLOW CYTOMETRY: TECHNICAL APPROACHES AND CLINICAL UTILITY

Sara Gutierrez Salamanca, Spain

Introduction: CAR-T cells (chimeric antigen receptor-T) therapy is an innovative immunotherapy developed for the treatment of different malignancies including lymphomas and leukemias, even though clinical trials with B-cell malignances are currently the most developed investigations, being CD19 the most explored target due to the high remission rates observed in treated patients (1). However, CAR-T cell expansion and persistence, after patient infusion, are critical factors to induce an efficient anti-tumor effect (2, 3), and flow cytometry becomes an essential tool for monitoring both in patients, being able to quantify the extent of CAR T cell expansion, and detect the presence of CAR T cells in peripheral blood (PB), bone marrow and cerebrospinal fluid (4, 5).

Methods and material: Flow cytometry use the capacity of the CAR molecule to bind its cognate antigen, coupled to a fluorochrome, to identify CART cells vs other immune cells. Based on that, an innovative universal CAR-T method of detection has been set up and validated to evaluate the expression of the functional CAR on the cell, in patients treated with different types of CAR-Ts cells aimed to CD19 and BCMA (patients= 70, samples= 640, CAR-T cells types= 7). This universal method of detection has been combined with the new tools that have been designed for detailed dissection of the different immune cell compartments in PB using automated 14-color 4-tube Next Generation Flow (NGF) approaches (6) and new spectral flow cytometers with >25 fluorescent detectors (Aurora, Cytek).

Results: This method offers a universal mean of detecting different types of commercial and academic CAR-Ts with the same target antigens and their combination with the 14 color 4-tube NGF approaches results in the identification of >250 leukocyte subsets in normal adult PB, including 85 subsets of CD4+ T-cells (e.g. 40 subsets of classical T helper cells, 25 distinct subpopulations of regulatory T-cells and 20 subsets of follicular T-helper cells with distinct functional roles), 48 subpopulations of CD8+ T- and NK-cells, 135 subsets of B-cells and plasma cells and 22 subpopulations of innate immune cells (www.EuroFlow.org) (6, 7), together with the detailed composition of the CAR-T population. NGF has the added advantage (versus other methods) of allowing a detailed composition of the CAR T population (identified with the adapted EuroFlow approach), in a short time (3 - 4 hours).

Conclusion: This method would allow to identify and predict relapses after CAR-T cell treatments, in multiple myeloma and B-non Hodgkin lymphoma patients. Besides, monitoring the CAR-T cells composition “ex vivo” contributes to understand the toxicity mechanism associated with CAR-T cells treatments and improve the clinical management of these patients.

WHY DO WE NEED CAR-Ts?

José Mário Mariz Porto, Portugal

Written summary not available.

OPENING CONFERENCE | ROOM 1

Light beyond the tunnel: Cytometry after horizon 2020

José Enrique O'Connor Valencia, Spain

For this closing lecture, I have chosen a title that wants to be a praise of the great momentum of flow cytometry evolution, driven by a constellation of intelectual and technological developments that has taken us from Cytology to Cytomics in less than 30 years. Currently, flow cytometry has become both a transversal technology and a promising businnes oportunity for large and small industries and biotechs. We were looking towards a promising horizon, when the dark times of COVID-19 came, and everything was arrested, and everybody was confined. However, among the many people, professional and not, that went out to help, cytometrist were in the front line very soon and very efficiently. Cytometry, as it happened after the onset of another terrible, still pandemic, AIDS, has provided crucial understading of the immunological basis fenomena underlying the cause and manifestations of COVID-19. We have learned many cytometric lessons because of AIDS, and we have applied them very rapidly to COVID-19. But, also, the cytometry community has wrapped together and has managed to maintain strong links along this period of physical distance, and this Virtual Congress is a wonderful example of how our community stays together for sharing knowledge and education, even in convulse times.

04. **ABSTRACTS**

ORAL PRESENTATION
INVITED ORAL PRESENTATION
E-POSTER
PUBLICATION

ABSTRACTS

ORAL PRESENTATION

TITLE ANTI-TRBC1 ANTIBODY-BASED DETECTION FOR ASSESSMENT OF T-CELL CLONALITY IN DIAGNOSTIC T-CELL FLOW CYTOMETRY PANELS: STANDARDIZATION OF SAMPLE PREPARATION AND ANALYSIS OF THE SPECIFICITY AND SENSITIVITY OF THE APPROACH **CODE** OP.01

AUTHORS NAMES Muñoz-García N.[1,2]; Mateos S.[1,2]; Caldas C.[1,2]; Balanzategui A.[2,3]; Alcoceba M.[2,3]; Morán F. J.[1,2]; Lima M.[4]; Villamor N.[2,5]; Orfao A.[1,2]; Almeida J.[1,2]

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ABSTRACT

Introduction: A single monoclonal antibody (TRBC1, clone JOVI-1) against one of two mutually exclusive T-cell receptor β chain constant region (TRBC) genes has been identified as a potential flow cytometry marker for clonality assessment. Our aim was to standardize the method for an appropriate routine use of anti-TRBC1 for clonality assessment and monitoring of clonal Tαβ cells, to establish its distribution in normal Tαβ-cell subsets according to the different TCRVβ families and to determine its sensitivity and specificity for detecting clonal T cell.

Methods: A total of 117 peripheral blood -PB- samples (76 from healthy donors -HD-, 10 from reactive polyclonal lymphocytosis and 31 from patients with T-cell chronic lymphoproliferative disorders -CLPD-) were included. Standardization of the TRBC1 staining procedure by flow cytometry was performed through comparison of 4 incubation conditions, to test the potential (stearic) interaction between TRBC1 and different CD3 conjugates: staining only with TRBC1 (clone JOVI-1), simultaneous staining with both antibodies, addition of TRBC1 10' before CD3 and addition of CD3 10' before TRBC1. The specificity of the approach was confirmed by the analysis of TRBJ1 or TRBJ2 genes by PCR on TRBC1+ and TRBC1- FACS-sorted cell populations, respectively. Conventional immunophenotyping approaches were also used to analyze the frequency of TRBC1+ cells within different (normal) T-cell subsets -according to the expression of 24 different TCRVβ regions- and to assess the sensitivity in detecting clonal cells -through real and virtual dilution experiments- and to validate it in T-CLPD.

Results: TRBC1 showed unspecific staining with high background in the absence of CD3, whereas TRBC1 labeling was significantly improved in the presence of CD3, particularly if the later antibody was added 10' after addition of TRBC1 (independent of the CD3 clone and the fluorochrome). Purified TRBC1+ and TRBC1- populations rearranged (at the DNA level) TRBJ1 in 25/26 Tαβ-cell populations (96%), and TRBJ1 plus TRBJ2 in 22/24 populations (92%), respectively, regardless of clonal status, confirming the specificity. In most TCRvβ families of Tαβ cells from HD, TRBC1/TRBC2 ratio was below 1, while significantly altered (vs. HD) in 9/24 TCRVβ families of reactive cases. Dilution experiments (of clonal cells in normal PB) showed that the sensitivity level for detecting clonal Tαβ cells (≥50 clustered events) was ≥10-4 in 7/8 T-CLPD cases tested. In all 31 T-CLPD in which the approach was validated, monotypic (monoclonal) expression of TRBC1 was confirmed.

Conclusion: Overall, the optimal TRBC1 staining is achieved when the antibody is added prior to CD3. Implementation of TRBC1 in flow cytometry is recommended as a fast, specific and accurate method for T-cell clonality assessment in diagnostic panels, as well as for detecting minimal (residual) disease, since a sensitivity level of ≥10-4 can be reached.

CONFLICT OF INTEREST No potencial conflict of interest to report.

TITLE AN ULTRA-RAPID FLOW CYTOMETRIC ASSAY FOR METHICILLIN RESISTANCE DETECTION AND VANCOMYCIN MIC DETERMINATION IN STAPHYLOCOCCUS AUREUS **CODE** OP.02

AUTHORS NAMES Rosário Gomes[1]; Sara Cruz[1]; Inês Martins-Oliveira[1]; Ana Silva-Dias[1,2]; Blanca Pérez-Viso[3]; Rafael Cantón[3]; Acácio Gonçalves Rodrigues[1,2] and Cidália Pina-Vaz[1,2]

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ABSTRACT

Introduction: Staphylococcus aureus is one of the most important human pathogens and there is a need of rapid susceptibility evaluation, especially methicillin-resistance detection (MRSA). Resistance to vancomycin, the main treatment of infections due to MRSA, is rare although, decreased susceptibility has been described and associated to worse outcome. A rapid antimicrobial susceptibility assay using flow cytometry to detect MRSA and the minimum inhibitory concentration (MIC) of vancomycin in S. aureus isolates is described.

Material and methods: Overall, 120 S. aureus isolates were investigated regarding methicillin-resistance using disk diffusion to cefoxitin and PCR for mecA detection; vancomycin MIC values were determined by broth microdilution (BMD) as well as population analysis profile for heteroresistance detection. In parallel, bacteria cells were incubated for one-hour with cefoxitin for MRSA detection and with a serial concentration of vancomycin for MIC determination together with a fluorescent probe that reveal membrane cell lesion. Flow cytometric analysis in the CytoFLEX from Beckman and a dedicated software was used to provide the susceptibility report. Categorical agreement was calculated regarding both drugs and Essential agreement regarding vancomycin comparing flow cytometric results with reference method.

Results: From the 120 S. aureus studied, sixty-five were classified as MSSA and fifty-five strains as MRSA by cefoxitin disk diffusion. Molecular assays found mecA gene in all MRSA studied and none of the MSSA strains. Distribution of MIC to vancomycin determined by BMD were between 0.25 and 8 mg/L, the upper range value due to the reference ATCC 700699-Mu50, GISA strain. According to the population analysis profiling, no heterorresistance was observed on studied strains as colonies were present in a maximum one dilution above MIC values.

Conclusion: The categorical agreement between the reference method and FC was 100% (CI 97-100%), regarding both drugs. Essential agreement regarding vancomycin MIC was 95.8% (CI 90.6- 98.2%). An ultra-rapid and accurate FC susceptibility assay is here described for methicillin-resistance detection and vancomycin MIC determination in S. aureus, yielding an excellent correlation with standard methods saving almost 1-day.

CONFLICT OF INTEREST No potencial conflict of interest to report.

TITLE MUTATIONAL PROFILE AND IMMUNOPHENOTYPIC CHANGES IN BONE MARROW COMPARTMENTS OF CHRONIC MYELOMONOCYTIC LEUKEMIA PATIENTS **CODE** OP.03

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ABSTRACT **Introduction:** Chronic Myelomonocytic Leukemia (CMML) is a heterogeneous disease, with overlapping features of myelodysplastic and myeloproliferative syndromes. Both immunophenotypic alterations and recurrent somatic mutations are found in CMML patients and can provide information on diagnosis and prognosis. We aimed to perform an immunophenotypic characterization of bone marrow compartments and its correlation with genetic alterations in a group of CMML patients (WHO2016).

Methods: Retrospective immunophenotypic analysis was performed according to EuroFlow panels and the Mean Fluorescence Intensity (MFI) from bone marrow (BM) granulocyte, monocyte and erythroid compartments of CMML patients (n=47) and from a normal BM (healthy group HG, n=16) was recorded. In 33 patients, targeted gene sequencing of 45 genes recurrently mutated in myeloid malignancies was performed on an Ion S5 (Thermo Fisher). Statistical analysis was done using SPSSv26.

Results: CMML patients were 70% male, median age 72y. WHO Classification: 42% CMML-0; 42% CMML-1; 16% CMML-2. FAB-Classification: 40% proliferative, 60% dysplastic. Mutations were identified in 97% of patients, with a median of 3 (0-7). ASXL1 (55%), TET2 (52%) and RAS pathway (22%) were the most frequently mutated genes. CPSS-Mol risk score was: 14% low; 20% int-1; 33% int-2; 33% high. Mutations in epigenetic modifiers genes alone (group A) were found in 21% and simultaneously with signal transduction genes (group B) in 36% of patients. Patients with mutated epigenetic modifiers had lower hemoglobin levels ($p<0.05$). We found an association between ASXL1 mutations and increased leukocyte count and between ASXL1 and RAS mutations with proliferative-CMML ($p<0.05$). Flow cytometry analysis of CMML BM revealed statistically significant differences in MFI in granulocyte (CD10, CD13, CD45), monocyte (CD11b, CD36, CD45, HLA-DR) and erythroid (CD45, CD105, CD117) maturation, compared with HG. Both groups A and B showed an increased MFI in CD45 in granulocyte and CD36, CD45, HLA-DR in monocyte maturation when compared to HG and increased CD11b MFI in monocyte maturation on group B. Decreased MFI in CD10, CD13 in granulocyte and CD45, CD105, CD117 in erythroid maturation also occurred in both groups. The more marked changes found in erythroid maturation in group A patients could be related to their lower haemoglobin levels, but further studies are required. We found no differences in MFI in the analysed markers among WHO classification or CPSS-Mol Score groups.

Conclusion: In CMML, there seems to be a specific immunophenotypic expression pattern in granulocyte, monocyte and erythroid maturation. Contrary to the remaining CMML BM, patients with mutations in epigenetic modifiers genes alone did not show a significant increase in CD11b expression in monocyte maturation. Detailed immunophenotyping of BM maturation compartments can further improve our understanding of CMML and help refine risk and prognosis classification.

CONFLICT OF INTEREST No potencial conflict of interest to report.

TITLE MONITORING THE IMMUNOPHENOTYPE DURING THERAPY WITH 5-AZACITIDINE MAY ASSIST IN REDEFINING THE QUALITY OF RESPONSE IN PATIENTS WITH HIGH-RISK MDS **CODE** OP.04

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ABSTRACT **Introduction:** In patients diagnosed with high-risk myelodysplastic syndrome (MDS) multiparametric flow cytometry immunophenotyping (FCI) identifies multiple abnormalities in several bone marrow (BM) cells lineages. 5-Azacitidine (AZA) remains as the standard of care for MDS patients with need of therapy. The quality of response to AZA is based on complex definitions involving hematological, morphological and cytogenetic data. This study investigated whether monitoring the FCI findings after AZA might offer any additional value over hematological information.

Methods: Five European Centers included 81 patients diagnosed with high-risk MDS (n=79) or chronic myelomonocytic leukemia (n=2) eligible for therapy with AZA. Each center processed its own BM samples and analyzed their FCI data comparing the findings in every single patient, before and after a median of 6 cycles of AZA (range 3-8 cycles). To normalize the results among centers, four possible definitions of FCI improvement were established based on the percentage and immunophenotype of the CD34 myeloid cells, granulocytic and monocytic patterns of maturation, erythroid cells abnormalities, granularity of granulocytes and percentage of monocytes. To summarize the results from the FCI and hematological responses, a square graphical representation was designed: lower left (LL) position for no hematological improvement (HI)/ no FCI improvement; upper left (UL) for cases with HI/no FCI improvement; upper right (UR) for HI & FCI improvement and lower right (LR) for no HI/FCI improvement.

Results: A good correlation between the hematological and the FCI response was observed in 53/78 patients with available data: 29 (37%) had HI & FCI improvement (UR) and 24 (31%) showed neither HI nor FCI improvement (LL). Twenty-two patients (28%) only showed HI (UL) and 3 (4%) only showed FCI improvement (UR). This distribution was similar irrespective of eligibility for stem cell transplantation (SCT). In order to determine any correlation between the position in the square and duration of AZA therapy, an additional analysis was performed on 52 patients not eligible for SCT. After a median of 6 AZA cycles, the square distribution for patients who finally received a maximum of 12 cycles of AZA was 3, 5, 11 and 1, as compared to 16, 9, 5 and 2 for patients who received >12 cycles of AZA ($P=0.01$). Among patients who achieved a HI after 6 cycles of AZA, the probability of maintaining this response at 12 cycles of AZA was twice as large (67%) for those patients who also showed FCI improvement after 6 cycles of AZA as compared to patients who did not (33%, $P<0.01$).

Conclusion: The combination of the FCI data and the hematological response after AZA can help clinicians to identify those MDS patients with a higher probability of maintaining a good quality of response for a longer period of time. This would prevent patients from unnecessary side-effects and might benefit from alternative therapeutic regimens.

CONFLICT OF INTEREST No potencial conflict of interest to report.

TITLE IMMUNOLOGICAL PARAMETER RECOVERY AFTER ACTIVE COVID-19 DISEASE **CODE** OP.05

AUTHORS NAMES Lamadrid-Perojo P.[1]; Renuncio-García M.[1,2]; González-López E.[1,2]; Roa-Bautista A.[1,2]; Gutiérrez-Larrañaga M.[1,2]; Guiral-Foz S.[1,2]; Merino-Fernández D.[1]; Alonso-Peña M.[1]; Iglesias-Escudero M.[1]; Irure-Ventura J.[1,2]; Comins-Boo A.[1,2]; López-Hoyos M.[1,2]; San Segundo D.[1,2]

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ABSTRACT **Introduction:** An inflammatory profile was identified in acute SARS-CoV-2 infection with lymphopenia, neutrophilia, disruption of Non-classical monocytes, increases in activated and exhausted phenotype of cytotoxic CD8+ T cells in addition to other parameters such as D-dymer, ferritin, serum interleukine-6, lactate dehydrogenase. However, the dinamics of immunological parameter has not been studied in detail. The present work assess the recovery of these parameters at short-term.

Material and methods: A total of 51 patients with active Covid-19 disease were recruited at admission in our Hospital, and subsequently studied at 7, 30 and 60 days. Only 5 out of 51 were admitted in Intensive care Unit, while within remaining 46, only 27 did not require oxygen therapy. A wide multiparametric panel was designed to study both innate and adaptive immune parameters, after staining with monoclonal antibodies the samples were adquired in Navios EX (Beckman Coulter) flow cytometer.

Results: An inflammatory profile in our cohort was confirmed. At admission the lymphopenia was confirmed at day 7 and a significant recovery at 30 days was observed (median 1078 vs 1152 vs 1831 vs 1688 a 0, 7, 30 and 60 days respectively Kruskall-Wallis (KW) p<0.0001). Likewise, the frequency of non-classical monocytes (CD14lowCD16++) was recovered at 30 days from admission (2.0 vs 1.5 vs 6.7 vs 11.7 KW p=0.0006). Similarly, a significant decrease in the frequency of cytotoxic NK cells (CD56lowCD16++) at first month from admission (96.7 vs 94.1, U-Mann Whitney; p=0.042) was observed. Finally, the frequency of activated cytotoxic T cells (CD8+CD38+HLADR+) was significantly increased after 7 days, and further decrease below basal levels at 30 days was observed (13.4 vs 22.8 vs 6.3 vs 6.5; KW p<0.0001).

Discussion: This work shows a recovery of inflammatory parameters at 30 days after admission independently of clinical severity. To assess the recovery of the inflammatory parameters associated with clinical outcome, larger studies with severe patients should be addressed. Funding: This work was partially supported by grant from Instituto de Salud Carlos III COV20/00170 and from Gobierno de Cantabria: 2020UIC22-PUB-0019

CONFLICT OF INTEREST No potencial conflict of interest to report.

TITLE DEEP IMMUNE CELL PROFILING IN BLOOD OF MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE (MGUS) **CODE** OP.06

AUTHORS NAMES Pérez-Escurza O.[1]; Pétursdóttir I.[2]; Flores-Montero J.[1]; Þórir J.[2]; Sanoja-Flores L.[1]; Damasceno D.[1]; Botafogo V.[1]; Morán J.[1]; Almeida J.[1]; Blanco E.[1]; Pérez-Andrés M.[1]; Pozo J.[1]; Durie B. G. M.[3]; van Dongen J. J. M.[4]; Yngvi S.[2]; Orfao A.[1]

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ABSTRACT **Introduction:** Multiple myeloma (MM) is a malignant neoplasm of terminally differentiated clonal plasma cells (PC) that typically accumulate in bone marrow (BM). From early stages of monoclonal gammopathy of undetermined significance (MGUS) to MM, the immune system plays a key role on disease onset and progression. Despite this, in depth characterization of immune cell profiles and kinetics at different stages of the disease remains to be fully investigated.
Aim: Here we evaluated the distribution of multiple immune cell populations in paired peripheral blood (PB) and BM samples of MGUS patients to identify altered immune cell profiles at the early stages of the disease.

Methods: Overall, we analyzed 50 paired PB and BM samples from 25 MGUS patients diagnosed in a screening study of the general population of Iceland (aged ≥ 40 y) in whom a monoclonal expansion was detected in serum. As controls, 64 PB samples from aged-matched healthy donors (HD) were studied in parallel. In each sample we determined the distribution of the major populations of myeloid cells -i.e. eosinophils, neutrophils, monocytes, basophils, dendritic cells, myeloid suppressor cells and CD34+ hematopoietic progenitor cells (HPC)- and lymphoid cells (i.e. B, T and NK), and their major functional and associated maturation subsets. In the patients, identification and characterization of clonal PC and B cells was also investigated in paired BM samples using high sensitive Next-Generation Flow Cytometry (NGF). For sample preparation, the EuroFlow antibody panels and protocols for NGF MM MRD and Immune monitoring were used.

Results: Based on WHO criteria, 21/25 patients were diagnosed with MGUS, 3 with SMM and 1 had MM. Clonal PCs were detected in BM of 22/25 (88%) cases, and clonal B cells were found in 3/25 (12%). Once the distribution of the distinct cell populations investigated in MGUS was compared to HD, differences were found for a total of 105 leukocyte cell subsets. Thus, MGUS patients showed significant decreased frequency of i) immature neutrophils, total dendritic cells, non-classical monocyte subpopulations; ii) regulatory CD4+ T cells; iii) several NK subsets, particularly the granzyme B+ ones; and iv) total, naïve and memory IgMD+ B cells. In contrast, MGUS patients had significantly increased frequencies of i) CD100+ DC; ii) CD4+ TFH cells with a regulatory T phenotype; iii) TCRgd and T CD8 cell subsets, particularly those with a granzyme-/CD57+ phenotype; and iv) subsets of CD21- memory B cells expressing IgG2, IgG4, IgA1 and IgA2. Most of these trends are also observed in SMM/MM. Once compared to MGUS, MM patients showed increased total effector memory TCD4+ cells and some subpopulations of TCRgd and IgA2+ memory B-cells.

Conclusions: Our results point out the existence of significant alterations in the distribution of immune cell populations in blood of MGUS and MM patients which might contribute to better understand the role of the immune system in PC neoplasm.

CONFLICT OF INTEREST No potencial conflict of interest to report.

TITLE DETECTION BY FLOW CYTOMETRY OF ALTERATIONS OF IMMUNE- AND PLATELET FUNCTION RELATED TO STRESS OR PATHOLOGIES IN MARINE MAMMALS **CODE** OP.07

AUTHORS NAMES Felipo-Benavent M.[1]; Martínez-Romero A.[1]; Rubio-Guerri, C.[2]; O'Connor J. E.[1]

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ABSTRACT

Introduction: It is well known that many pathologies may alter immune or platelet functionality. Some infections trigger inflammatory processes that activate immune cells, while others, such as viral infections, can cause immunosuppression. Besides, weakening of the immune function can foster the appearance of other pathologies. If the pathology involves hemorrhages or thrombocytopenia, we can also observe changes in the platelets function. On the other hand, stress is an important predisposing factor for immunosuppression. Furthermore, adrenaline is considered as a platelet agonist, so stress can lead to an enhancement of platelet function. Immune and platelet functions are not assessed in routine veterinary controls, and much less in marine mammals. However, applying flow cytometry (FCM) to monitor such parameters systematically may be of interest to detect diseases early, thus improving both prevention and monitoring.

Methods: Our group has adapted existing FCM protocols to assess phagocytic capacity (Ingoflow and Fagoflow Kits, Exbio), as well as developed in-house techniques to measure leukocyte bioenergetics, platelet activation and immature platelet levels in marine mammals. In the last years, blood samples of healthy animals were taken periodically to determine the physiological values of each parameter in several marine mamals species. When some animal displayed infections or hemostasia disorders, the immune and platelet functions have been evaluated with the indicated FCM assays, to detect possible alterations.

Results: We present several clinical cases that exemplify the efficacy of these FCM methods to detect alterations in immune or platelet function previously or during various diseases. Dolphins with acute or chronic respiratory or urinary infections presented an exaggerated activation of phagocytic cells compared to their companions. In contrast, a dolphin suffering a respiratory viral disease exhibited reduced phagocytic capacity, as is typical in certain virus infections. On the other hand, chronically stressed dolphins presented recurrent immunosuppression, hemorrhagic gastritis and a higher platelet activation comparing with the average. Furthermore, animals with slight to severe hemorrhage (gastrointestinal or bite wound) also presented active platelets and an increase of immature platelets level in blood. Interestingly, a routine examination detected immunosuppression in a dolphin that after a few days began to show symptoms of fungal infection.

Conclusion: The adapted FCM methods for immune and platelet monitoring are useful for the early diagnosis of certain diseases as well as for evaluating possible alterations derived from them in marine mammals. Mar Felipo-Benavent is the recipient of a Predoctoral Research Contract (ACIF) of the Generalitat Valenciana.

CONFLICT OF INTEREST No potencial conflict of interest to report.

TITLE BONE MARROW FOLLICULAR-LIKE T CELLS IN MONOCLONAL GAMMOPATHIES **CODE** OP.08

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ABSTRACT

Introduction: Monoclonal gammopathies (MG) comprise a group of disorders including monoclonal gammopathy of undetermined significance (MGUS), smoldering multiple myeloma (SMM) and symptomatic multiple myeloma (MM), which result from the proliferation of clonal plasma cells (PCs). Lymphoplasmacytic lymphoma (LPL) is a low-grade B-cell lymphoma characterized by small B-lymphocytes that may exhibit plasmacytoid or plasma cell differentiation. Waldenström's Macroglobulinemia (WM), which accounts for 95% of all LPL cases, is defined by bone marrow (BM) infiltration of B cells and PCs, and the presence of a monoclonal immunoglobulin M (IgM) protein, regardless of the size. The triggering event that drives tumorigenesis in MG and LPL/WM is speculated to happen in the germinal center (GC), which depends on follicular-like helper T-cells (TFH). Therefore, we aimed to analyse different follicular-like T cell subpopulations in the BM microenvironment of MG and LPL/WM patients to hypothesize if these cells continue to support GC reactions after disease development.

Material and methods: We evaluated bone marrow samples from 7 normal subjects (control group); 21 from MM precursor diseases (MGUS and SMM); 20 from MM and 9 from LPL/WM patients. Through multiparameter flow cytometry we analysed follicular like T cells among CD4+, CD4+ regulatory (regs), CD8+, CD8+ regs, γδ+, CD4-CD8- αβ+, CD4+CD8+ and CD4+CD8+ T regs cells. Follicular-like T cells were identified based on the expression of CXCR5, and activated T cell subsets according to CD25 and HLA-DR expression.

Results: Concerning the frequency of follicular-like T cells among the T cell subpopulations under study, we only observed an increased frequency of CXCR5+CD8+ T regs in all patient groups, in comparison to controls. When evaluating if follicular-like T cells exhibit an activated phenotype, based on CD25 or HLA-DR expression, we detected a significant increase of activated follicular like CD4+, CD4-CD8- αβ+ and CD4+CD8+ T cells in the LPL/WM group. However, activated follicular like CD4-CD8- T cells were found to be significantly decreased in MG patients, albeit more pronounced in the MM group, when compared with normal controls and LPL/WM.

Conclusion: To our knowledge, this is one of the first studies analysing TF cells in the tumour microenvironment of patients with MG and LPL/WM. Our results uncover an engagement of bone marrow follicular-like T cell subsets, particularly those that exhibit an activated phenotype in LPL/MW and not in MG. Furthermore, an increased frequency of activated CD4+ Tregs cells was observed in all patient groups, particularly those with MM and LPL/MW.

CONFLICT OF INTEREST No potencial conflict of interest to report.

TITLE ACUTE LEUKEMIA IN A 1 DAY-OLD NEWBORN **CODE** OP.09

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ABSTRACT **Introdution:** During the first year of age, the appearance of infant acute leukemia and transient abnormal myelopoiesis is often associated with Down syndrome (DS). Transient abnormal myelopoiesis (TAM) of DS occurs in approximately 10% of DS neonates and in phenotypically normal neonates with trisomy 21 mosaicism. This clonal disease characterized by immature megakarioblasts is a preleukemic syndrome. TAM generally regresses spontaneously without any treatment within the first few months of life. However, some patients might need a low dose of chemotherapy to increase survival rate and prevent other morbidities like hepatic or multi-organ failure. Despite its typical transient nature, 20% to 30% of patients with TAM develop overt acute leukemia, usually within 3 years. We present a case of an acute leukemia in a 1 day-old newborn with a DS-like phenotype.

Methods: A 43-year-old caucasian woman, aparently healthy, presented to the emergency room of the hospital in labour. It had been an unknow and unattended pregnancy. The delivery was uneventful, and an aproximatly 37 week baby girl was born with DS phenotype. The complete blood count revealed a slight anemia with a high corpuscular volume, thrombocytopenia, and an elevated white blood cell count, with the presence of 20% blast cells. In the peripheral blood smear there were very basophilic blast cells, with cytoplasmatic blebs, nucleated red blood cells and megakaryocyte fragments. The remaining biochemistry revealed elevated uric acid and liver enzymes, with a markedly high LDH. The phenotypic study of peripheral blood by immunophenotyping and the karyotype were immediately sent to an external institution.

Results: The immunophenotyping report revealed the presence of 21,4% of blast cells, with an with heterogeneous expression of CD36 and CD71. A low positive expression was observed for CD45, CD42a+CD61 and CD123. Partial positive expression in CD34, HLA-DR, CD117, CD7, CD36, CD71, CD33, CD38, CD42b, CD41B and CD9. CD56, with partial expression, was present as an aberrant marker. A negative expression was observed for CD13, CD16, CD10, cMPO, citoCD79A, CD19, CD3, citoCD3, CD35, CD14, CD300e, CD64, CD105, CD22, 7.1(NG2), CD15, CD203c, CD25 and nTdT. From this it was asserted the presence of 21,4% of myeloid line blasts with maturation to the megakaryocytic line, suggestive of acute megakaryoblastic leukemia. The DS karyotype was confirmed.

Conclusion: Pregnancy surveillance has allowed for a drastic reduction in maternal, fetal and infant morbility and mortality. Clinical pathology can play an important role in the monitoring of all the above stages, and its predictive to have trained and experienced professionals in detecting and alerting for urgent situations. While the reported case might prove to be a transient leukemia, vigilance of the newborn's stability is still predictive, thus obliging the necessity of a very close follow-up.

CONFLICT OF INTEREST No potencial conflict of interest to report.

TITLE EXPERIMENTAL AND DATA PROCESSING WORKFLOW FOR LARGE-SCALE IMMUNE MONITORING STUDIES BY MASS CYTOMETRY **CODE** OP.10

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ABSTRACT Mass cytometry is a powerful large-scale immune monitoring technology. It requires a careful experimental and analytical design to ensure a maximal data quality. Here we present an experimental protocol for whole blood analysis together with an r-based data analysis pipeline which ensures the minimization of the experimental artifacts and batch effects, while ensuring data reproducibility. Firstly, we have evaluated two whole blood preservation protocols that allow rapid sample processing and long-term stability: Phosphoflow Fix and Lyse (BD) and Proteomic Stabilizer (PROT) using an 8–plex panel for conventional flow cytometry (FC) and a 26-plex panel for mass cytometry (MC). Manual gating of circulating leukocyte populations and cytokines was performed. We observed high correlation and low bias towards any cell population when comparing fresh and 6 months frozen blood with FC and MC. Low coefficients of variation (CV) across studied time points indicate good sample preservation for up to 6 months. Cytokine staining stability was also confirmed. Additionally, we tested the stability of a single sample over a 13-month period using 45 consecutive aliquots and a 34-plex panel by mass cytometry, showing remarkable sample stability. Secondly, we designed a protocol for large-scale functional cytometry studies on whole blood samples, together with an R-based automated data analysis pipeline, which ensures the minimization of experimental artifacts and batch effects, while optimizing data reproducibility. Whole blood samples are stimulated, fixed and frozen before barcoding together with a reference sample, and afterwards are stained for subsequent acquisition. Data preprocessing and quality controls are carried out using an R pipeline and packages like CATALYST for bead-normalization and debarcoding, flowAI and flowCut for signal anomaly cleaning, AOF for files quality control, flowclean and flowDensity for gating, CytoNorm for batch normalization and UMAP for data exploration. This protocol is particularly suitable for large-scale, multicenter, multibatch and retrospective studies.

CONFLICT OF INTEREST No potencial conflict of interest to report.

TITLE FLOWCT FOR DECONVOLUTION OF LARGE IMMUNOPHENOTYPIC DATASETS AND BIOMARKER DISCOVERY IN CANCER IMMUNOLOGY **CODE** OP.11

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ABSTRACT **Introduction:** Large-scale immune monitoring is becoming routinely used in clinical trials to identify determinants of treatment responsiveness, particularly to immunotherapies. Flow cytometry remains one of the most versatile and high-throughput approaches for single-cell analysis, but manual interpretation of multidimensional data poses a challenge to capture full cellular diversity and provide unbiased reporting.

Methods: We present FlowCT, a semi-automated workspace empowered to analyze large datasets that includes pre-processing, normalization, multiple dimensionality reduction techniques, automated clustering and predictive modeling tools. As a proof of concept, we used FlowCT to unbiasedly compare the T cell compartment in bone marrow (BM) vs peripheral blood (PB) of patients with smoldering multiple myeloma (MM); identify minimally-invasive immune biomarkers of progression from smoldering to active MM; define prognostic T cell subsets in BM of active MM patients after treatment intensification; and determine the longitudinal effect of maintenance therapy in BM T cells. A total of 290 BM and PB samples from 250 patients were included in this study and those with active MM were treated according to the PETHEMA/GEM2014MAIN clinical trial.

Results: FlowCT starts by creating a matrix with expression data and follows with data quality control and normalization (eg, gaussNorm, Harmony or canonical correlation analysis). Automated clustering can be performed using numerous methods (FlowSOM, Phenograph, PARC or Seurat) before dimensionality reduction to visualize clusters' identity before manual annotation (within FlowCT or using other flow cytometry software). Sub-clustering of a desired cell population can be iteratively performed. Different tests to evaluate statistical correlations or differences across groups as well as machine learning algorithms to predict outcomes are available in FlowCT. While performing an objective comparison of lymphocyte distribution in PB and BM of SMM patients, FlowCT enabled the identification of 25 T cell subsets including unique phenotypic and transcriptional states within the CD4 differentiation and polarization trajectories. By applying the gradient boosting algorithm to the datasets of patients with smoldering and active MM, we respectively identified immune T-cell signatures of malignant transformation (CD4+CD28negTIGIT+CD127lo, CD4+CD28+TIGIT+CD127+, CD4+CD28+TIGIT+CD127+CD25+, CD8+CD28negCD127+, CD8+CD28negTIGIT+ and CD8+CD28negTIGIT+PD1+; hazard ratio [HR]: 7.33, P = 0.002) and inferior survival (CD4 and CD8 double positive, CD4 naïve, Treg CCR7neg and CCR7+, CD8 TCM CD127lowPD1het and CD8 TEMRA CD127lowPD1+; HR: 7.60, P = 0.0022).

Conclusion: FlowCT is a new open-source computational approach that can be readily implemented by research laboratories to perform quality-control and analyze high-dimensional data, unveil cellular diversity and unbiasedly identify biomarkers in large immune monitoring studies.

CONFLICT OF INTEREST No potencial conflict of interest to report.

TITLE URINE CYTOMETRY FOR NON-INVASIVE DIAGNOSTIC OF RENAL PATHOLOGY ASSOCIATED TO SYSTEMIC AUTOIMMUNE DISEASES **CODE** OP.12

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ABSTRACT **Introduction:** In systemic autoimmune diseases (SADs), nephritis is one of the most severe manifestations and it can only be assessed by renal biopsy. However, the invasiveness of this diagnostic technique makes it unsuitable for early detection of renal pathology or to monitor the response to treatment. Currently, the monitoring of renal inflammatory activity is carried out using serum and urinary analytical parameters that are neither very specific nor able to differentiate renal activity from chronicity. Therefore, there is a real need to establish new biomarkers for patients at risk of suffering from renal pathologies associated to SADs. Renal pathologies associated to SADs are due to local immune response, and this may be reflected in the composition of the urinary sediment and the surface antigen expression profile of urinary exosomes. Therefore, urine can directly reflect the real-time inflammatory status of the kidney. Thus, this study aims to use urine as a potential non-invasive source of information about renal pathology associated with SADs.

Methods: Samples of urine from patients diagnosed with SADs were analysed. Six groups of diseases were included: systemic lupus erythematosus (SLE), Sjögren's syndrome (SjS), rheumatoid arthritis (RA), scleroderma (SSc), primary anti-phospholipid syndrome (PAPS), mixed connective tissue disease (MCTD) and undifferentiated connective tissue disease (UCTD). We used flow cytometry to analyse the expression of 37 markers in surface of exosomes and to characterize the immune cells populations in the urine sediment.

Results: We found a significant increase of HLA-ABC, CD63 and CD105 expression in surface of exosomes from patients with nephritis compared to non-nephritis patients, as well as a higher expression of surface exosomal marker CD81 in SLE and SjS patients with clinical parameters of renal damage. On the other hand, while migrant CD11b+ neutrophils were found increased in SLE patients, monocytes and CD4+ T cells were reported to be higher in SjS patients compared to controls. Finally, different populations of dendritic cells (DC), such as plasmacytoids DC and CD1c+ DC, were observed in the urine of SADs patients, and their numbers were significantly greater compared to healthy donors.

Conclusion: Results of this study showed that the differential expression of exosomal surface markers may be helpful in the diagnosis of nephritis in SADs patients and to stratify patients according to renal damage. During pathology development, an infiltrate of immune cells populations occurs in the urine of these patients, consequence of the renal chronic inflammation processes that take place. Nevertheless, longitudinal studies with patients with renal involvement under treatment are needed to confirm the possible use of urine as source of information for the diagnosis of renal pathology associated to SADs.

CONFLICT OF INTEREST No potencial conflict of interest to report.

ABSTRACTS

INVITED ORAL PRESENTATION

IOP.01 HARACTERIZATION OF INTRATUMORAL INNATE LYMPHOCYTE POPULATIONS

Margareta Correia Porto, Portugal

The immune system is classically divided into innate and adaptive. In the last decade, the family of innate lymphocytes has been growing. Besides natural killer (NK) cells, which are cytotoxic, it is now known that there are different subsets of so called innate lymphoid (ILCs). ILC1s, ILC2s, ILC3s are mainly tissue resident and are cytokine producers per definition, mirroring their T helper counterparts from the adaptive system. ILCs are described to play important roles in immune responses against extracellular pathogens and tissue homeostasis. However, their putative role in cancer remains poorly explored. Here we isolated immune cells from hepatocellular carcinoma (HCC) samples and analyzed intratumoral ILCs, with a particular focus on ILC3s, providing novel insights on their putative anti-tumoral immune responses.

IOP.02 UNRAVELLING THE BLADDER TUMOUR MICROENVIRONMENT USING THE FLOW CYTOMETRY TOOLBOX

Andreia Peixoto Porto, Portugal

Bladder cancer constitutes one of the deadliest genitourinary diseases, especially when diagnosed at late stages. These tumours harbour microenvironmental niches characterized by low levels of oxygen (hypoxia) and limited glucose supply due to poor vascularization. However, the synergic contribution of these features to disease development is poorly understood. This presentation focuses on the flow cytometry guided study of bladder cancer cells phenotypic changes promoted by hypoxia and glucose shortage. Accordingly, we demonstrated through Annexin V/PI-double-staining that bladder cancer cells are significantly plastic in adapting to microenvironmental changes in nutrient availability, resisting nutrient suppression as well as reoxygenation and restored access to glucose without triggering stress-induced apoptosis. Cell cycle analysis has confirmed a delay or arrest in S/G2 transition under microenvironmental pressure, which was consistent with reduced cell proliferation and chemoresistance in vitro. Glycomics and FACS analysis have confirmed a major antagonization of O-glycosylation pathways, leading to simple cell glycophenotypes characterized by the accumulation of immature short-chain O-glycans at the cell surface. Glycoengineered models reflecting simple cell glycophenotypes were developed and validated by FACs. Functional studies in vitro and in vivo showed that Tn and STn short-chain O-glycans overexpression decreased proliferation and promoted chemoresistance, reinforcing their close link with tumour aggressiveness. Collectively, flow cytometry approaches have contributed to the demonstration that hypoxia and glucose deprivation trigger more aggressive cell phenotypes, promoting what appears to be an escape mechanism from microenvironmental stress. We propose that, altered glycosylation may be used to target these subpopulations, paving the way for precision oncology.

IOP.03 THE APPLICATION OF FLOW CYTOMETRY IN TESTING NEW THERAPEUTIC STRATEGIES IN PRECLINICAL IN VIVO MODELS

Carlos Palmeira Porto, Portugal

Over the last decade, based on a growing understanding of cancer biology and an extensive development of preclinical animal models and clinical trials, the efficacy and mechanisms of immunotherapy have been fully explored. Significant and lasting clinical response with Immunotherapy provide a new breakthrough treatment for a variety of refractory cancers.

Among the different types of cancer Immunotherapy strategies, Immune-Checkpoint Inhibitors (ICIs) are one of the most studied and used in the clinic.

Although Immune-Checkpoint inhibitors drugs are promising for achieving longer-term efficacy, the frequency of patients reaching these types of response is very low. Most of patients do nor response or inevitably develop resistance to treatment after a period. And this resistance may occur through different and complex mechanisms.

It is therefore necessary to develop new therapeutic strategies, namely by combining immunotherapies with therapies that target cancer cells but stimulate the immune system. The idea that these therapies can stimulate the immune system is because when they targeted tumour cells, they cause apoptosis and necrosis of these cells, leading to the release of tumour antigens. This tumor cell death is associated to an acute inflammatory response that has been proven to enhance the immune response.

Two of these therapies are Photodynamic therapy (PDT) and Interstitial Laser Thermotherapy (ImILT). However, the synergistic effect of these therapies and immunomodulator therapy (like Immune -checkpoint inhibitors) has not been investigated, and therefore, the aim of this project is to evaluate the combination effect of these therapies with anti-PD-1 and anti-CTLA-4 therapies, regarding treatment response. In this preclinical model, female C57BL/6 mice, inoculated with a mouse melanoma cell line, B16F10, are used. This tumour cell line was chosen because it is poorly immunogenic and we intend to evaluate the possibility of reversing this characteristic with combination therapies.

Here some of the preliminary results obtained by flow cytometry analysis are presented. So far, when therapies were combined it was observed: an increase in cytotoxic T cells, with an activation phenotype and some memory phenotype; and a decrease in regulatory T cells, i.e. immunosuppressive cells. These results suggest that the synergistic effect of these therapies allows a local immune response, and this can make it possible to reverse the poor immunogenic feature of this tumour model.

IOP.04 THE POTENTIAL OF EXTRACELLULAR VESICLES CARGO AS MOLECULAR BIOMARKERS FOR CLEAR CELL RENAL CARCINOMA PATIENT'S MANAGEMENT

Francisca Dias Porto, Portugal

The definition of molecular biomarkers of cancer recurrence is essential to do a more precise and individualized patients' follow-up. The advantages of studying the extracellular vesicles content for cancer patient's management and as a mirror of the tumor microenvironment is an opportunity for a more accurate patients' stratification using minimally invasive methods, such as liquid biopsies. This lecture explores the applicability of the mRNA and miRNA content of plasmatic EVs from clear cell Renal Cell Carcinoma patients as new prognostic biomarkers.

ABSTRACTS

E-POSTER

TITLE A RAPID FLOW VIROMETRIC TEST FOR DETECTION OF SARS-COV-2 PARTICLES **CODE** B.01

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ABSTRACT **Introduction:** Coronavirus disease 2019 (COVID-19), caused by infection of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), is a major health issue declared as pandemic in March 2020. The early detection of infection is essential to minimize the magnitude of widespread disease dissemination. According to the World Health Organization (WHO), the gold standard method to detect SARS-CoV-2 is RT-qPCR. Rapid Antigen Detection (RAD) test is less time consuming and has less sensitivity. In this study, we have developed a rapid and sensitive flow virometric assay aimed at SARS-CoV-2 detection, based on RNA staining, global protein content and light scatter properties of virus particles.

Methods: SARS-CoV-2 virus particles were obtained from the supernatant of infected Vero E6 cells under High biocontainment (IRTA-CReSA ABSL3 facilities). As a negative control, supernatants of non-infected Vero E6 cells were used. Clarified supernatants were inactivated with 4% paraformaldehyde and 1% glutaraldehyde to get better particle structure preservation. Samples were stained with Hoechst 33342 (DNA), Pyronin Y (RNA) and FITC (global protein) for 15min at 4ºC, protected from light. Negative and positive controls were acquired and analyzed on the Attune™ NxT Flow Cytometer (Thermo Fisher). Hoechst 33342, Pyronin Y and FITC were excited at 405, 561, and 488 nm respectively. Analysis for SARS-CoV-2 spike S protein was performed using anti-SARS-CoV-2 spike protein recombinant human monoclonal antibody (Thermo Fisher).

Results: Small particle analysis of supernatants obtained from non-infected Vero E6 cells showed no signal for RNA. Samples containing virus particles showed specific signal for Pyronin Y. RNA/FITC positive particles were negative for Hoechst 33342, showing light scatter properties compatible with viral particles of sizes less than 200 nm. The S protein of SARS-CoV-2 was confirmed after staining with anti-SARS-CoV-2 spike protein recombinant human monoclonal antibody.

Conclusion: This 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 will help to confirm or rule out 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.

CONFLICT OF INTEREST No potencial conflict of interest to report.

TITLE DETECTION OF EXOSOMES BY CONVENTIONAL FLOW CYTOMETRY: PROTOCOL OPTIMIZATION **CODE** B.02

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ABSTRACT **Introduction:** The study of extracellular vesicles, in particular exosomes, in the context of disease biomarkers has become increasingly relevant in many fields. Exosomes are membrane-enclosed micro- and nano-sized vesicles containing bioactive molecules that can carry and transfer molecular messages. Their lipid bilayer membrane confers stability and prevents their cargo - DNA, messenger RNA (mRNA), microRNA (miRNA), proteins, cytokines – from degradation during the circulation throughout biological fluids (such as blood, urine, and saliva). Exosomes play an important role in cell-to-cell communication, influencing both physiological and pathological processes. Exosomes can either be up-taken by neighbouring recipient cells, where they release their content, or can navigate through the body to reach distant organs. Thus, exosomes allow us to indirectly detect signs of changes in cellular function by studying easily obtained samples such as peripheral blood. The aim of this work was to optimize a protocol to detect exosomes by conventional flow cytometry.

Methods: Through a sequential ultracentrifugation, we isolate exosomes secreted by AML cell line models – HL-60, NB-4 and KG-1. After, a physical characterization was performed by nanoparticle tracking analysis (NTA), to confirm the vesicles size and concentration. Next, using these samples we applied a strategy to detect exosomes by conventional flow cytometry. For that, we stained the exosomes with the surface markers CD38 and CD73. Then, we separate CD38+ CD73+ from CD38- CD73- by cell sorting, followed by immunoblotting analysis, to confirm the presence of vesicles.

Results and conclusion: Herein, we present a conventional flow cytometry to analyse extracellular vesicle, particularly exosomes. This research was funded by FEDER and Foundation for Science and Technology (FCT), grant number POCI-01-0145-FEDER-028159 and POCI-01-0145-FEDER-030782 BSM and A.C.A were founded by FCT, grants number DL 57/2016 and POCI-01-0145-FEDER-028159, respectively. Conflicts of Interest: The authors declare no conflict of interest.

CONFLICT OF INTEREST No potencial conflict of interest to report.

TITLE FLOW CYTOMETRY AS A TOOL TO CHARACTERIZE AN ENGINEERED 3D IN VITRO MODEL OF ACUTE MYELOID LEUKEMIA

CODE B.03

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ABSTRACT **Introduction:** Acute myeloid leukemia (AML) is the most common acute leukemia, characterized by an impaired hematopoesis affecting the production and normal function of blood cells. Adult hematopoiesis takes place in the bone marrow (BM), where hematopoietic stem cells (HSCs) can self-renew, proliferate and differentiate to replenish the blood and immune systems [1]. HSCs acquisition of somatic mutations, accompanied by BM microenvironmental alterations are reported to be involved in the development of AML. However, it remains unclear what are the mechanisms involved in the clonal survival. Furthermore, due to the BM location and its complex nature, it remains difficult to identify such mechanisms. Here we developed a 3D in vitro leukemic model, capable of reproducing important features of the BM such as the three-dimensionality of the extracellular matrix and the physical interaction between leukemic and stromal cells [2]. Characterization of the 3D engineered model was performed by flow cytometry allowing single cell analysis. We believe that developing such model will allow us to investigate the mechanisms that facilitate the clonal expansion.

Methods: Briefly, cell laden collagen type I hydrogels were used to culture individually or simultaneously, Hl-60 cells, a widely used AML cell line, and mesenchymal stem cells. As control, gold standard 2D cultures were used. At different time-points (3 and 6 days), engineered constructs were enzymatically digested with collagenase to liberate the cells. Flow cytometry was used to characterize cell viability (Annexin/Propidium iodide (PI) staining), cell cycle (PI), and mesenchymal stem cell differentiation (surface markers CD73, D90, CD105, lack of expression of CD34 and CD45). Morphological characterization was performed by confocal microscopy.

Results: Our results showed that culturing cells in the 3D engineered system does not compromise cell viability when compared to controls. The percentage of necrotic, late apoptotic, early apoptotic and healthy cells was not significantly different between controls and 3D cultures at day 3 and 6. Furthermore, cell cycle showed no significantly differences between controls and 3D cultures at day 3. However, while in controls at day 6 the percentage of cells in G1 phase increased, in the hydrogel this percentage was similar for both time-points. Furthermore, engineered 3D system seems to promote the formation of leukaemia- cells colonies without any additional stimuli.

Conclusion: Our 3D engineered model showed to be suitable to culture BM cells that could potentially be used to study mechanisms of clonal expansion. Funding: POCI-01-0145-FEDER-028159 and POCI-01-01 [1] Morrison, S.J. and D.T. Scadden, The bone marrow niche for haematopoietic stem cells. Nature, 2014. 505(7483): p. 327-34. [2] Aleman, J., et al., Deconstructed Microfluidic Bone Marrow On-A-Chip to Study Normal and Malignant Hemopoietic Cell-Niche Interactions. Small, 2019. 15(43): p. e1902971.

CONFLICT OF INTEREST No potencial conflict of interest to report.

TITLE BORING BEADS NO MORE! SIMULTANEOUS IMAGING AND ANGLE-RESOLVED SCATTERING OF ANISOTROPIC PARTICLES IN FLOW

CODE B.04

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ABSTRACT **Introduction:** Imaging flow cytometry combines the high information content of microscopic images with the high throughput of flow cytometry. Possible applications include the diagnosis of acute myeloid and lymphoid leukemia in immunophenotyping, detection of rare circulating tumor cells (CTC) in liquid biopsy, or analysis of morphological cell changes like mitosis. The discrimination of single cells, coincidences, and agglomerates presents a challenge in common flow cytometry. Counting rare blood cells like CTCs in whole blood significantly raises the chance for counting errors due to coincidences. We overcome this challenge by expanding a laser flow cytometer with multi-dimensional imaging capabilities to improve the discrimination of above events and thus the precision of cell concentration measurements. Our goal is to develop a new reference method to support external quality assurance, thereby enhancing the precision, reproducibility, and comparability of clinical measurements. Our method facilitates personalized medicine - ultimately saving lives.

Methods: Our setup consists of two lasers with wavelengths of 406 nm and 488 nm. After elliptical shaping, they are focused into a flow cell. The sample is delivered by a motorized syringe pump. We detect light scatter in forward (FSC) and sideward (SSC) direction, and six fluorescence signals with photomultiplier tubes (PMT). Two additional industrial CMOS cameras capture images of the SSC's spatial distribution and fluorescence, and of the FSC's angular distribution (Fourier plane). A beam stop blocks the laser beams in forward direction. The 488 nm laser is vertically offset to act as a trigger for the cameras and the probe laser, where the trigger window is set on one of the PMT signals. Each signal within the lower and upper limit of the trigger window generates a trigger signal. The camera is triggered directly, whereas the probe laser triggers with a configurable time delay (µs) and duration (ns) to account for the flow speed. This way, although the cameras' exposure times of >60 µs exceed the laser-object interaction time, sharp images are generated. Numerical simulations of the generalized Lorenz-Mie scattering theory and simulated imaging accompany the measurements to support data interpretation.

Results: From the fluorescence images, we can directly access particle dimensions such as diameter and count all visible objects. The angular-resolved FSC and spatial-resolved SSC signals create specific structures, which we can compare with the simulations, e.g. to analyze the geometry of agglomerates. This way, we can efficiently differentiate single cells from agglomerates and coincidences.

Conclusion: We have successfully shown the capability of industrial CMOS cameras to produce FSC, SSC, and fluorescence images. Our setup enhances a conventional laser flow cytometer with the possibility of multi-dimensional imaging of objects to improve the counting accuracy of cell concentration measurements.

CONFLICT OF INTEREST No potencial conflict of interest to report.

TITLE HEMOPHAGOCYTIC SYNDROME–ASSOCIATED INTRAVASCULAR LARGE B-CELL LYMPHOMA: A CHALLENGING DIAGNOSIS **CODE** H.01

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ABSTRACT

Introduction: Intravascular large B cell lymphoma (IVLBCL) is a rare and aggressive subtype of extranodal lymphoma in which the proliferation of neoplastic cells occurs almost exclusively within the lumen of capillaries. There are three main variants: classic, cutaneous and associated with hemophagocytic syndrome (HPS).

Methods: Presentation and discussion of a clinical case.

Results: A 37-year-old woman without relevant personal and family medical history was referred to our hematology consultation in April 2020 with B symptoms, bicytopenia and splenomegaly. Blood tests revealed normocytic normochromic anemia with hemoglobin (Hb) 8.3g/dL, platelets 85x109/L, monocytes 0.97x109/L, lactate dehydrogenase (LDH) 794U/L (normal: <214), ferritin 1114ng/mL (normal: 15-150) and C-reactive protein (CRP) 6.1mg/dL (normal: <0.5). The peripheral blood (PB) smear was normal. Infections and autoimmune diseases were exhaustively excluded. CT scan showed splenomegaly of 26cm. PB and bone marrow (BM) flow cytometry (FCM) ruled out acute leukemia and lymphoproliferative disorders. The BM aspirate and biopsy revealed only a marked hypercellularity. Meanwhile, the patient experienced clinical and analytical worsening with Hb 6.9g/dL, platelets 90x109/L, monocytes 6.25x109/L, LDH 857U/L, ferritin 4010ng/mL, triglycerides 438mg/dL (normal: <150). A second BM evaluation revealed reactive plasmacytosis and diagnostic splenectomy showed reactive red pulp hyperplasia. To confirm/rule out autoimmune lymphoproliferative syndrome and HPS, a PB cytometry was performed, which showed increased α/β+ CD4-CD8- T cells (7.0% of T cells), T cell activation, sharp expansion of pro-inflammatory (CD14+dimCD16+) monocytes (53% of monocytes), and a marked elevation of plasma IL-10. NGS for gene mutations in FAS, FASLG and CASP10 was negative. In July 2020, large aberrant cells, likely activated monocytes/macrophages, were observed in the PB film. At this point, new BM and PB FCM studies confirmed the expansion of pro-inflammatory monocytes and revealed, for the first time, rare large B cells with aberrant immunophenotype and DNA aneuploidy (0.3% leukocytes). Positive markers: CD45dim, CD5, CD19, CD20bright, CD30dim, CD38dim, CD40bright, CD86, FMC7, HLA-DRbright. Negative markers: CD10, CD15, CD23, CD25, CD34, CD56, CD79b, CD95, CD138, CD200, and immunoglobulin light chains. CT scan showed massive hepatomegaly. IVLBCL was later diagnosed by histological assessment of liver biopsy. The patient started chemotherapy with R-CHOP.

Conclusion: This case highlights the glitches associated with IVLBCL diagnosis, which are related to its rarity, poorly understood pathophysiology and highly heterogeneous presentation, and emphasizes the vital role of FCM in identifying the neoplastic B cells and confirming macrophage activation. Increased plasma IL-10 levels found in IVLBCL have been attributed to IL-10 production by neoplastic B cells.

CONFLICT OF INTEREST No potencial conflict of interest to report.

TITLE IMMUNOLOGIC CHARACTERIZATION OF CORONAVIRUS DISEASE 2019 (COVID-19) PATIENTS WITH HEMATOLOGICAL CANCER: BIOLOGIC AND CLINICAL SIGNIFICANCE **CODE** H.02

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ABSTRACT

Introduction: The COVID-19 pandemic is having a profound impact in oncologic care. Recent analyses suggest that hematological cancer patients may have an increased risk of severe complications, including death, due to myelo- and lympho-suppression caused by cancer itself and cytotoxic treatment. Thus, greater knowledge on the immune status of hematological patients may be useful to optimize prevention, risk stratification and treatment strategies.

Methods: We used multidimensional flow cytometry and a semi-automated pipeline to analyze immune profiles in peripheral blood (PB) samples of 513 COVID-19 patients at presentation and 167 during follow-up. Furthermore, in 14 cases we performed a deep immunophenotyping of lymphoid cells and transcriptome analysis of six FACSorted myeloid- and dendritic-cell (DC) subsets.

Results: Of the 513 COVID-19 patients, 10 had hematological tumor. These patients showed a similar frequency of hospitalization compared to those without an active tumor (80% vs 77%), but a significantly higher frequency of intensive care unit admission (50% vs 5%) and death from COVID-19 (30% vs 4%). Hematological patients displayed altered immune profiles with significantly decreased percentages of classical monocytes, immunoregulatory NK-cells, double-positive T-cells and mature B-cells when compared to those without tumor. RNAseq data from basophils, myeloid and plasmacytoid DC, classical and non-classical monocytes and neutrophils showed considerable clustering of hematological cases. Genes related to NF-κB and STAT transcription factors as well as genes encoding toll-like receptors and proinflammatory interleukin receptors, all of which implicated in the response and evasion of innate sensing by coronaviruses, were differentially expressed between COVID-19 patients with and without blood cancer. Deep phenotypic characterization of lymphoid cells showed that the relative distribution of antigen-dependent maturation stages within the T-cell compartment was generally similar between both groups, but significantly altered in several B-cell subsets, such as those expressing IgG and IgA subclasses. During follow-up, there was a profound variation from the first to the latest PB sample in the relative distribution of all immune cell-types in COVID-19 patients bearing a hematological tumor when compared to other cases. Although the immune kinetics were quite variable, cancer patients dying from COVID-19 tended to have increased numbers of neutrophils counterbalanced by reduced percentages of other cell-types vs those who survived.

Conclusion: Our study exposes that hematological patients show a constellation of immune alterations that could compromise the response to SARS-CoV-2 infection, suggesting an association between impaired immune responses and poorer outcomes in COVID-19 patients with hematological malignancies.

CONFLICT OF INTEREST No potencial conflict of interest to report.

TITLE FLOW CYTOMETRY DETECTION OF SUSTAINED HUMORAL IMMUNE RESPONSE (IGG+IGA) IN ASYMPTOMATIC/ MILD SARS-COV2 INFECTION **CODE** H.03

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ABSTRACT

Introduction: In the last months it has been noted the need for improved specificity and sensitivity in classic serological techniques. In this regard, flow cytometry (FCM) represent a promising tool, since it allows the detection of antibodies against the native and functional S-protein of SARS-Cov-2 exposed into the membrane of a transfected cell line, simulating the physiological conditions.

Methods: Analysis of 50 samples from qPCR+ patients, gathered between November-December, 2020 and 50 pre-pandemic samples (negative controls) collected between May-June, 2019. Median time elapsed from first qPCR+ was 249 days (range 220-271). Samples were also tested by a commercial chemiluminescence immunoassay (CLIA). Finally, we performed a functional analysis for the assessment of neutralizing antibodies. We have based our technique on the one described by Horndler et al12. For each individual test, we used a mixture of 25% of wild type Jurkat cells and 75% of transfected Jurkat cells (S-Jurkat), expressing the S-protein and EGFR (as control of transfection) for the study of the presence of polyclonal antibodies against the S-protein. The samples were acquired in an Omnicyt flow cytometer (Cytognos,S.L). IgG and/or IgA antibodies specifically bound to S-proteins were identified through the comparison of the median fluorescence intensity (MFI) of the S-Jurkat and the wild type Jurkat cells in each sample. Besides, we studied the correlation between the expression of IgG/ EGFR and IgA/ EGFR using a linear regression analysis. All the experiments were analyzed using the Infinicyt 2.0 software (Cytognos,S.L.).

Results: MFI-ratio in pre-pandemic and SARS-CoV-2 samples Pre-pandemic samples showed an IgG-MFI ratio of 1.18 (95%CI 0.96 to 1.40) and IgA-MFI ratio of 1.12 (95%CI 1.0 to 1.35). It was not observed a significant linear correlation between IgG/EGFR and/or IgA/EGFR. (R2 0.1). All samples of PCR+ patients showed an IgG-MFI ratio ≥ 1.4 (mean= 4.12, range= 1.54-7.11) and positive IgG/EGFR correlation, thus were considered anti-S/IgG+. IgA was positive in 88% (44/50) of samples (IgA-MFI ratio ≥1.35; mean= 2.30, range= 1.5-7.8) using the MFI ratio method. Comparison with CLIA and functional analysis Discordant results were observed in 6 patients (5 FCM+ /CLIA-; 1 FCM- /CLIA+). Those samples were subjected to a functional assay to evaluate the presence of neutralizing antibodies, which was confirmed only in the FCM+ /CLIA- cases. We could not demonstrate the presence of neutralizing antibodies in the FCM- ELISA+/CLIA+ case.

Conclusion: This strategy confirms that FCM is a highly specific and sensitive technique for the detection of neutralizing antibodies against SARS-Cov-2. FCM constitutes a promising tool to look at long-term protective humoral immune response in cases where antibody levels were predictably low, as in the long-term monitoring of asymptomatic patients, immunosuppressed individuals', or elderly patients.

CONFLICT OF INTEREST No potencial conflict of interest to report.

TITLE ALTERED PHENOTYPIC AND FUNCTIONAL IMMUNE PROFILES IN SYSTEMIC MASTOCYTOSIS **CODE** H.04

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ABSTRACT

Introduction: Systemic Mastocytosis (SM) is a heterogeneous disease characterized by the presence of KIT D816V mutation in most (>90%) of the cases. This mutation promotes a constitutive activation of tumour mast cells (MC) that triggers the release of mediators, leading to an altered immune microenvironment with important consequences on innate immune cells, particularly on monocytes and dendritic cells (DCs).

Objective: To investigate the potential existence of an altered distribution and functional behaviour of blood circulating innate immune cells other than MC and its association with distinct subtypes of the disease.

Methods: Immunophenotypic characterization was carried out for the identification of different subsets of blood circulating monocytes and dendritic cells. Studies for Ex vivo and in vitro cytokine production, quantification of soluble cytokine levels and CCL2/MCP-1 and EMR2 plasma levels were also performed. In total, around 114 patients, including 44 indolent SM (ISM) patients without skin lesions (ISMs-), 62 ISM patients with skin lesions (ISM+) and 8 patients with aggressive SM forms (ASM), and 21 healthy adults (HA) were analysed.

Results: SM patients showed lower counts than HA, at the expense of recently produced (CD62L+/FcεRI+) classical monocytes in ISMs+ patients. This increase is followed by decreased intermediate and non-classical monocyte (ncMo) counts in SM compared to HA. Regarding DCs, ISMs+ and ASM patients showed decreased numbers at the expenses of both plasmacytoid DCs and myeloid DCs, with an expansion of the minor population of AXL+ DCs in ISMs-. In addition, increased spontaneous cytokine production of IL1β, IL6, IL8 and TNFα is associated with an exhausted ability of LPS + IFNγ blood monocytes from SM patients to produce inflammatory cytokines compared to HA. Plasma levels of inflammatory cytokines were higher in SM patients compared to healthy adults.

Conclusion: SM patients displayed re-distribution of blood monocyte and DC subsets, suggesting innate cells other than mast cells may also play a critical role in the pathophysiology and clinical manifestations of mastocytosis. In addition, blood monocytes from SM patients are constitutively activated and functionally exhausted, and contribute, at least in part, to the increased plasma levels of a wide variety of inflammatory cytokines.

CONFLICT OF INTEREST No potencial conflict of interest to report.

TITLE ANGIOIMMUNOBLASTIC T CELL LYMPHOMA: MULTIPARAMETER FLOW CYTOMETRY AS A USEFUL DIAGNOSTIC TOOL. A REVIEW OF A CASE SERIES IN OUR DEPARTMENT **CODE** H.05

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ABSTRACT **Introduction:** Angioimmunoblastic T-cell lymphoma (AITL) is a neoplasm of mature T follicular helper (TFH) cells that accounts for 1-2% of all non-Hodgkin lymphomas. AITL is a very aggressive disease and diagnosis requires positive immunostainning for at least 2 of the following 7 antigens: CD10/BCL6/PD1/CXCL13/CXCR5/ICOS/SAP and immunophenotypic aberrancies of pan-T markers. In a few cases, the diagnosis remains challenging by morphology and immunochemistry (IHC), and multiparameter flow cytometry (MFC) can be useful as it is a sensitive procedure that allows identifying small populations of aberrant T-cells in a background of reactive and inflammatory cells.

Methods: In this review, we describe three cases with confirmatory diagnosis of AITL by MFC, morphology and IHC (cases 1-3), and two cases (4 and 5) with immunophenotype suggestive of AITL by MFC, but discordant by IHC. MFC was performed with FACSCanto II (BD Biosciences) through Infinicyt™ software (Cytognos) and it was based in Euroflow protocol.

Results: In our case series, the three AITL confirmatory diagnosis present CD3- CD4+ pathologic T-cells, showing the utility of CD3-/CD4+ gate. In this way, in case 1, a population of T cells with aberrant immunophenotype sCD3- CD4+ was detected in PB and LN. Case 2 supports versatility of MFC and utility of CD3-/CD4+ gate, when this approach allowed to detect AITL lymphocytes in various samples just as LN, AF and BM. Case 3 proves that FC is also useful in the follow up, since at diagnosis it detected 1,5% of aberrant T cells in BM, and it showed subsequent relapses. Regarding CD10 expression, case 1 did not express CD10, case 2 showed dim CD10 expression and case 3 had bright CD10 expression. CD10 expression by FC has a high sensitivity, but it's less specific to differential diagnosis from other PTCL. Histologically, several cases of AITL may mimic Hodgkin lymphoma. For instance, in case 4, MFC in LN, PB and BM was suggestive of AITL. However, the diagnosis was challenging by histology, finally concluding Hodgkin lymphoma. Similarly, there are cases of AITL that show a reactive hyperplasia pattern and may be misinterpreted as a benign process. In case 5, MFC detected clearly a 1,8% of aberrant lymphocytes suggestive of AITL and the patient had skin rash, eosinophilia and lymphadenopathy. Histological diagnosis suggested dermatophatic lymphadenopathy, and unfortunately the patient died in another institution by disease progression without treatment.

Conclusion: We emphasize the useful and versatility provided by MFC for the immunophenotypic diagnosis of AITL in different samples and with a high sensitivity in detecting aberrant T-cells population, even if this population represents a low percentage of the total celullarity. Our experience show that histological confirmation is not easily achieved in several cases and in this way, MFC is a fast, reproducible and highly sensitive tool.

CONFLICT OF INTEREST No potencial conflict of interest to report.

TITLE VALIDATION OF PLATELET CD34 EXPRESSION FOR THE SCREENING OF CONGENITAL MACROTHROMBOCYTOPENIA ASSOCIATED WITH MUTATIONS OF THE GFI1B GENE **CODE** H.06

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ABSTRACT **Introduction:** Growth factor-independent 1B (GFI1B) is an important transcription factor for megakaryocyte development and acts as a negative regulator for CD34 expression. Previous studies have identified GFI1B as a causative gene for a rare form of autosomal dominant congenital macrothrombocytopenia (MTP) associated with variable α-granule deficiency and bleeding tendency. GFI1B variants cause heterogeneous phenotypes dependent on the site of mutation. While platelet (PLT) counts, PLT granule defects and severity of bleeding vary in affected individuals with distinct GFI1B variants, CD34 expression in PLT seems to be a consistent feature in all cases. We validated CD34 expression in PLT for the screening of congenital MTP associated with GFI1B mutations (MTP-GF1B).

Methods: We studied 3 patients with GFI1B-MTP and 5 patients with MTP with other genetic defects. Simultaneously with each patient, we studied 2-4 healthy controls (HC) (blood donors). Eighteen additional HC were studied, totalizing 32 HC. Platelet CD34 expression was evaluated in whole blood anticoagulated with sodium citrate and stained with anti-CD34 PerCPCy5.5, -CD41a FITC and -CD42b PE, using a NAVIOS flow cytometer. The median fluorescence intensity (MFI) of CD34 expression was corrected for PLT size using the forward scatter (FSC). To evaluate the intermediate precision (IP), 3 PB samples from HC were evaluated simultaneously by two operators. To assess intra-operator repeatability (IOR), 3 PB samples from HC were processed by the same operator in duplicate. The interclass correlation coefficient (ICC) and coefficient of variation (CV) were calculated. Sensitivity and specificity were used to assess test performance.

Results: Controls (n=32): The median and P5-P95 values of the CD34-MFI were 1.110 (100%) and 0.555-1.429 (50%-129%), respectively. GFI1B-MTP (n=3): CD34-MFI were 1.590 (152%), 2.470(197%) and 4.390(421%), respectively, as compared to a median value of 1.184 (0.920-1.380) observed in 8 HC studied in parallel. Non-GFI1B-MTP (n=5): CD34-MFI were 0.602(56%), 0.662(61%), 0.862(69%), 0.438(35%) and 1.563(126%), respectively, as compared to a median value of 1.192(0.953-1.260) observed in 6 HC studied in parallel. IP: The ICC was 0.9874 for individual values and 0.9937 for average values (excellent repeatability). The CV was acceptable (9.3%). IOR: The ICC was 0.9996 for individual values and 0.9998 for average values (excellent repeatability). The CV was acceptable (0.3%). Test performance: CD34-MFI >129% (P95) were obtained in all GFI1B-MTP patients and none of the patients with non-GF1B-MTP, suggesting a sensitivity and specificity close to 100% for the diagnosis of GFI1B-MTP.

Conclusion: According to these preliminary results, obtained with a limited number of cases, CD34 expression in PLT is a common feature of perturbed GFI1B function, and may have utility for diagnosis of GFI1B-MTP. It is necessary to study more patients to confirm the performance of the test.

CONFLICT OF INTEREST No potencial conflict of interest to report.

TITLE PRIMARY MEDIASTINAL LARGE B CELL LYMPHOMA: A CASE REPORT **CODE** H.07

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ABSTRACT **Introduction:** Primary mediastinal large B-cell lymphoma (PMLBCL) is an uncommon lymphoma that represents 6-10% of all diffuse large B-cell lymphomas. It typically manifests with a bulky anterior mediastinal mass with a local invasion tendency leading to compression symptoms. Although PMLBL is a distinct clinicopathological entity in the WHO classification of lymphoid malignancies, its features can overlap with thymic neoplasms and other B-cell lymphomas. In many cases, diagnosis is delayed by the challenge to obtain representative biopsy samples.

Methods: We describe a case of PMLBCL, in which flow cytometry (FCM) made an important contribution to the diagnosis.

Results: A 25 years-old man presented with a 1-month history of asthenia and tiredness associated with episodes of chest pain radiated to the back that worsened with inspiration and cough and improved by leaning forward. In the previous two weeks, he also had an engorged neck and dry cough. Physical examination showed jugular vein distension, tachycardia, hypophonic heart sounds and vesicular breath sounds. There were no peripheral adenopathies, hepatomegaly, or splenomegaly. Chest computed tomography (CT) scan revealed an anterior mediastinal mass (8.1x7.6x11.1cm) with compression of the left brachiocephalic and superior cava veins. There were several small pulmonary nodules up to 2 cm in size, and a nonspecific nodular image with 3 cm in diameter in the liver. Endobronchial ultrasound-guided transbronchial needle aspiration of the mediastinal mass gave inadequate material for cytological analysis. However, FCM identified 1% of large abnormal B-cells, which were positive for CD19, CD20high, CD10low CD23high, CD38low, CD79high, CD81high, CD200high, FMC7high, BCL2, and kappa light chains and negative for CD5, CD25, CD30, CD38, CD56, highly suspicion of PMLBCL. Then, a core biopsy was performed, and histopathological examination showed diffuse proliferation of large B-cells with abundant cytoplasm, oval to irregular nuclei with distinct nucleoli, associated with sclerosis, thereby confirming the diagnosis. After 6 courses of R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone), the patient achieved clinical remission. CT showed densification in the pre-vascular space, but no adenomegalias or other relevant findings. Treatment proceeded with consolidation radiotherapy. After one month, there were no hypermetabolic changes in the positron emission tomography.

Conclusion: PMLBCL represents about 10% of all mediastinal neoplasms in adults, but the only way to differentiate it from the others is through the immunophenotype and histological evaluation. This case illustrates the importance of acquiring representative sampling to diagnosis mediastinal masses. With a thorough sample of the mass, FCM was able to identify rare neoplastic B cells with immunophenotypic features typically observed in PMLBCL. The adequate treatment was initiated, allowing the patient to recover.

CONFLICT OF INTEREST No potencial conflict of interest to report.

TITLE A NEW SCORE TO PREDICT OUTCOME IN ACUTE MYELOID LEUKEMIA **CODE** H.08

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ABSTRACT **Introduction:** Alkaline phosphatase (ALP) is an enzyme highly expressed in primitive stem cells and its activity is altered in leukemia, among other disease conditions. We previously studied ALP activity in human myeloid leukemia in combination with immunophenotyping (LG Rico et al., 2019). Our previous findings suggested that increased cellular alkaline phosphatase activity in leukemic cells at diagnosis was significantly associated with a higher risk of relapse, treatment resistance, and mortality. ALP activity quantification was determined as the percentage of ALPhigh cells within the leukemic blast population. The aim of the present study was to generate a more objective and standardized method to quantify cellular ALP activity related to estimate the probability of relapse, disease persistence and overall survival more accurately.

Methods: A novel ALP score was calculated as the ratio between the ALP median fluorescent intensity (MFI) from blasts and lymphocytes. In order to verify the new score, we compared the outcome and the event-free survival (EFS) and the overall survival (OS) in n=42 AML patients, using data previously obtained by LG Rico et al., 2019. Flow data was obtained with the Attune™ Nxt Flow Cytometer (Thermo Fisher) and analyzed using Infinicyt™ Software v.2.0.4.b.000 (Cytognos, S.L.).

Results: Accordingly to the new ALP score, AML patients were classified into three risk groups: favorable (ALP score < 1.20), intermediate (1.20 1.83), and adverse (ALP score > 1.83). Differences in outcome were analyzed and compared with the percentage-based ALP score (LG Rico et al., 2019). Four patients in favorable group achieved complete response (100%), showing significant differences with intermediate and adverse groups (p-value = 0.002; 95% CI = 0.00 – 1.00). Nineteen patients in adverse group relapsed or showed treatment resistance (86%) in contrast with 11 patients from intermediate and favorable groups (52%) (p-value = 0.02; 95% CI = 0.03 – 0.90). EFS was significantly shorter in the adverse group, when compared with the favorable group (4 months versus not reached, p-value = 0.01). OS was shorter in the adverse group, when compared with the favorable group, with no significant differences (11 months versus not reached, p-value = 0.09).

Conclusion: Comparison of the ratio- and the percentage-based ALP scores suggest that the ratio-based score can be used to better predict event-free survival in AML. Importantly, the ratio-based score provides a more objective and standardized method to analyze ALP activity. Despite the cohort's small number, significant differences were obtained for both ALP scores, suggesting that ALP activity is associated with the potential risk of recurrence and mortality in newly diagnosed patients, as well as to the clonal diversity of primitive ALP+ stem-like subsets in AML.

CONFLICT OF INTEREST No potencial conflict of interest to report.

TITLE T CELL PROLYMPHOCYTIC LEUKEMIA DIAGNOSED IN THE PRECLINICAL PHASE BY FLOW CYTOMETRY: THREE CLINICAL CASES **CODE** H.09

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ABSTRACT **Introduction:** T cell Prolymphocytic Leukemia (T-PLL) is a rare and clinically aggressive lymphoproliferative disorder. Most patients are adults and have advanced disease, with marked lymphocytosis, cytopenias, adenopathies, hepatosplenomegaly and, sometimes, skin lesions and serous effusions. The small cell T-PLL variant has better prognosis. Flow cytometry (FCM) is important for diagnosis. Most cases are CD4+/CD8-, some are CD4+/CD8+, and few are CD4-/CD8+. TCL1 expression is usually observed, because most common genetic alterations involve chromosome 14 (14q32.1), conditioning activation of the TCL1 proto-oncogene; deletions or inactivating mutations of the tumor suppressor ATM gene (11q22.3) may also occur. Ataxia telangiectasia (AT) is a rare primary immunodeficiency resulting from germline biallelic ATM mutations, causing genome instability. Secondary genetic events, such as TCL1 rearrangements, are frequent in AT patients. These patients have neuronal degeneration, immunological abnormalities, and increased risk of developing cancers, including T-PLL. We describe three patients with T-PLL diagnosed in the preclinical phase, two of them with AT.

Methods: Clinical and analytic evaluation of three cases of T-PLL diagnosed pre-clinically, including blood counts, lymphocyte morphology, T cell immunophenotyping by FCM, TCRB rearrangements by PCR, and cytogenetic analysis by FISH.

Results: One patient (male, 59 years-old) was referred by lymphocytosis diagnosed in routine blood analysis, and two patients (one female and one male, 24 and 27 years-old), were referred for lymphocyte immunophenotyping as part of the routine monitoring of a previously diagnosed AT, with biallelic ATM mutations. FCM allowed for diagnosis of T-PLL: the abnormal T cells were positive for CD2low, CD3, CD5high, CD7high, CD28, and TCL1, among others; one case was CD4+CD8- and two cases (both with AT) were CD4+CD8-/+low. Lymphocyte morphology was compatible with small cell variant T-PLL. All patients had TCL1 rearrangements and the non-AT patient had del11q22.3. At physical examination, none of the patients had adenopathy, splenomegaly, hepatomegaly or serous effusions. Both AT patients had cerebellar ataxia and cutaneous-mucous telangiectasias, and one had a history of recurrent infections. Blood counts were normal in AT cases and revealed slight lymphocytosis in the non-AT patient. Lactic dehydrogenase and B2-microglobulin were normal. AT patients had increased alpha-fetoprotein. After a median follow-up of 19 months, both AT patients remain stable, without disease manifestations and the non-AT patient experienced disease progression at 16 months, needing for treatment with alemtuzumab (19 months).

Conclusion: These cases highlight the problems associated with T-PLL diagnosis in the preclinical phase, needs of disease monitoring and concerns in deciding the best time to start treatment, which in AT patients poses additional challenges due to the risk of toxicities.

CONFLICT OF INTEREST No potencial conflict of interest to report.

TITLE CLONAL CIRCULATING PLASMA CELLS – WHEN THE DIAGNOSTIC CRITERIA FOR PLASMA CELL LEUKEMIA ARE NOT MET **CODE** H.10

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ABSTRACT **Introduction:** Plasma Cell Leukemia (PCL) is an aggressive plasma cell (PC) disorder characterized by the presence of clonal PC >20% of leukocytes in peripheral blood (PB) or absolute count >2000/L (Kyle's criteria)1. This definition includes both primary PCL — in the absence of previous Multiple Myeloma (MM)—and secondary PCL - leukemic transformation of MM2. Primary PCL is a distinct clinical entity characterized by a younger age, rapid progression and poor prognosis. In the current definition of PCL, patients with circulating PC < 20% are classified as MM, however, recent studies have shown that MM patients with either ≥ 2% or ≥ 5% circulating PC in PB have adverse outcomes in PCL, suggesting that a lower threshold should be used.3,,4,5

Methods: Case report

Results: Male, 45 years, ECOG 0, presents with 10% (1100/uL) aberrant PC in PB and no lytic lesions. Cytogenetic analysis showed no apparent high-risk abnormalities although the status of t(14;16) wasn´t studied. Medullary histology had extensive PC infiltration, which represented about 40-50% of the cell population. Flow cytometry (FC) analysis showed the presence of 16.4% of pathologic PC in PB and 23.2% in bone marrow with immunophenotype: CD19-, CD20-, CD27-, CD28+, CD33-, CD34-, CD38++, CD45-, CD56-, CD81+, CD117-/+, CD138++ and cyKAPPA . Since the morphological criteria for PCL were not met, a diagnosis of IgG-Kappa MM, ISS-3, was assumed. Bortezomib, Thalidomide and Dexamethasone (VTD) followed by stem cell transplantation was proposed. Due to renal failure at diagnosis, five hemodialysis sessions were performed, with improvement. After the third VTD, due to disease progression, the regimen was changed to Daratumumab, Thalidomide, Cyclophosphamide and Dexamethasone.

Conclusion: In light of current knowledge, immunophenotypically, both in PCL and MM, CD38 and CD138 are positive and CD2, CD3 and CD16 consistently negative. The markers that differ in their expression are CD20, CD56, CD9, CD117 and HLA-DR.1,5,6,7,8 PCL tend to express a more immature phenotype, and show a higher expression of CD20 and lower HLA-DR compared to MM. 1,5,6,7,8,9 In addition, PCL is marked by the absence of CD33 and CD56.1,6,7,8,10,12 Of the requirements defined for the diagnosis of PCL, both IMWG and WHO suggest that one of the Kyle's criteria must be present.8,12 However, threshold established was arbitrary and several studies have questioned this cutoff,7,13 even IMWG in 2013 suggested that this criteria should be revised.12 A study using FC came to the conclusion that the number of circulating PC is an independent prognostic factor and that scores ≥ 400 pathological PC in 150,000 events (0.26% circulating cells) leads to a significant reduction in overall survival.10 This case emphasizes the importance of reviewing the diagnostic criteria for PCL and finding a new threshold through more sensitive, accurate and reproducible technology, such as FC, thus improving the diagnosis and risk stratification.

CONFLICT OF INTEREST No potencial conflict of interest to report.

TITLE MALIGNANT PLEURAL EFFUSION AND TRANSFORMED LYMPHOMA **CODE** H.11

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ABSTRACT Pleural effusion is a common complication of non-Hodgkin and Hodgkin lymphomas, occurring in up to 30% of cases, and adversely affecting overall survival. However, in Waldenström's Macroglobulinemia (WM), a rare indolent B-cell lymphoproliferative disorder characterized by bone marrow infiltration of clonal lymphoplasmacytic cells that produce monoclonal IgM paraprotein, pulmonary involvement, including pleural effusion, occurs in 50g/L) was identified by serum immunofixation. WM was, therefore, diagnosed. A right thoracoscopy with subsequent pleurodesis was performed revealing multifocal nodular lesions of the pleura, which were biopsied. The involvement of the pleura by a DLBCL was documented. The patient started RCHOP chemotherapy, directed against the aggressive lymphoma. Subsequent molecular studies confirmed the same clonal IGH and IGK rearrangements in both lymphoproliferative diseases, thus implying lymphomatous transformation. Although an undetermined complete remission was achieved after 6 RCHOP cycles, the patient relapsed in less than 2 years, synchronously from the DLBCL and the lymphoplasmacytic lymphoma/WM, detected through a lymph node and skin lesion biopsies, respectively. In conclusion, this rare case of transformed lymphoma, with an atypical clinical presentation, portrays the pivotal role of FCI in the management of patients with malignant pleural effusion. For hematolymphoid disorders, FCI of diverse body fluids is a rapid, reproducible, sensitive, and quantitative method, proven to be extremely useful both in the setting of a known disease and de novo lymphoma diagnosis.

CONFLICT OF INTEREST No potencial conflict of interest to report.

TITLE PLATELET SIZE EVALUATED BY THE FORWARD SCATTER IN INHERITED THROMBOCYTOPENIAS **CODE** H.12

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ABSTRACT **Introduction:** Inherited thrombocytopenias (IT) are heterogeneous disorders whose knowledge has greatly increased in recent years. The diagnostic algorithm presented in 2013 by Balduini et al. considers that, once excluding the syndromic forms, the platelet (PLT) size is important to guide the diagnosis. However, the correct assessment of PLT size is not easy, because the mean platelet volume measured with automated electronic counters frequently underestimates it, especially in cases of thrombocytopenia with very large PLTs. To minimize this problem, we evaluated the possibility of using the PLT Forward Scatter (FSC), obtained when performing the determination of the PLT glycoproteins (GP) by flow cytometry (FC), to estimate the PLT size, and investigated it´s value to identify different groups of IT, including inherited macrothrombocytopenia (IMT) and IT with normal PLT size.

Methods: We analyzed the PLT-FSC, using a Navios flow cytometer, in 71 patients with IMT – 8 MYH9-related thrombocytopenia (RT), 17 Bernard Soulier syndrome (BSS), 29 ITGA2B/ITGB3-RT, 11 ACTN1-RT and 6 TUBB1-RT – and 10 patients with IT with normal PLT size - all with ANKRD26-RT - diagnosed in our center. We compared the patients´ PLT-FSC with the median PLT-FSC value obtained in four healthy controls (HC) (blood donors) studied in parallel (313 HC in total), and calculated the FSC index (% normal values) for each patient, as follows: patient PLT FSC / median PLT-FSC value obtained in HC*100. The results were expressed as median, minimum and maximum values. Differences between groups were evaluated using the Mann Whitney test. For test performance, we calculated the sensitivity, specificity, positive and negative predictive values (SS, SP, PPP and NPP) of FCS indexes above 175% and above 250% for specific IMT subtypes.

Results: PLT-FSC: PTL-FSC from patients with MYH9-RT, biallelic-BSS, ACTN1-RT, ITGA2B/ITGB3-RT, and monoallelic-BSS, were significantly higher ($p<0.001$ in all cases) and PTL-FSC from patients with TUBB1-RT were slightly higher (p0.05). PLT-FSC indexes: The following FSC indexes were obtained – MYH9-RT: 339% (197-545); Biallelic BSS: 200% (165-258); ACTN1-RT: 146% (132-176); ITGA2B/ITGB3-RT: 135% (76-175); Monoallelic BSS: 127% (108-167); TUBB1-RT: 125% (117-143); ANKRD26-RT: 90% (79-126). Test performance: PLT-FSC indexes >175% had a SS of 95%, SP of 99%, PPP of 95% and NPP of 99% for the diagnosis of MYH9-RT or biallelic BSS, which can be easily distinguished based on the GPIB/V/IX levels. FSC indexes >250% had a SS of 88%, SP of 100%, PPP of 100% and NPP of 99% for diagnosis of MYH9-RT.

Conclusion: We confirmed the importance of PLT size in the algorithm of investigation of IT, and validated the utility the PLT-FSC to measure it by FC. The FSC index allows to estimate variations in PLT size and to discriminate some IMT with high sensitivity and specificity.

CONFLICT OF INTEREST No potencial conflict of interest to report.

TITLE NODAL GAMMA-DELTA T CELL LYMPHOMA – CASE REPORT **CODE** H.13

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ABSTRACT **Introduction:** Approximately 10 to 15% of non-Hodgkin's lymphomas (NHL) derive from T or NK cells and only 2-4% of T-cell lymphomas express gamma delta (γδ) T Cell Receptor. The World Health Organization (WHO) classification recognizes four subtypes of γδ-T lymphoid neoplasms: hepatosplenic γδ T-cell lymphoma, primary cutaneous γδ T-cell lymphoma, monomorphic epitheliotropic intestinal T-cell lymphoma and large granular lymphocytic leukaemia. These neoplasms are very rare and aggressive, and their diagnostic may be very difficult.
Complication of NHL include pleural, pericardial or peritoneal effusions. The cytological and immunophenotypic examination of these fluids may be useful for the diagnosis and classification of the lymphoma.

Case report: A 75-year-old woman, with no relevant medical history, was admitted to the hospital due to abdominal pain and B symptoms. She presented mild thrombocytopenia, with no other relevant alterations in the peripheral blood count. A computed tomography scan revealed numerous peripancreatic, retroperitoneal and intraperitoneal lymphadenopathies, splenomegaly, pleural effusion and ascites. The analysis of the pleural fluid showed a predominance of lymphocytes with 12% large mononuclear cells, with basophilic cytoplasm and reticulated chromatin. The immunophenotypic characterization by flow cytometry revealed 14% of large cells expressing: CD3+ CD4- CD8++hom CD2++hom CD5++hom CD7++ CD1a- CD11b- CD11c+hom CD14- CD16- CD26- CD27+ CD28+ CD30- CD45RA- CD45RO+ CD56- CD57- CD94- CXCR4- CCR7+ CD279- Granzyme+ Perforin+ HLADR+ TCL1- TCRγδ+ and TdT-. A lymph node biopsy was performed. The immunohistochemistry study showed T cells CD3+ CD5+ CD2+ CD4- CD8+ Bcl2+ Bcl6+ MUM-1+ CD20- pax5- CD10- CD30- CD15- and ALK-. A Ki67 of 90% was estimated.
Bone marrow biopsy showed involvement by the T cell neoplasm, with an interstitial pattern. Flow cytometry of the peripheral blood revealed 2.5% of neoplastic cells.
Cytogenetic analysis was not performed.
The patient initiated treatment with CHOP, but following the 3rd cycle she experienced a worsening of her clinical condition due to disease progression.

Conclusion: Although the WHO classification is helpful in defining the γδ T-cell lymphoma subtypes, there are rare variants described in literature that are not included in the current version of WHO classification, which may hamper their diagnosis and treatment. The case reported herein may correspond to one of these rare variants, the nodal γδ T cell lymphoma. It's a rare lymphoma with disseminated nodal involvement, with variable cell morphology and frequent bone marrow infiltration. Hepatosplenomegaly may be present. This subtype is very resistant to chemotherapy and has a poor prognosis.
Gamma-delta T-cell lymphomas are still a poorly understood pathology due to their low incidence. The report of these rare cases may help clinicians to better understand, diagnose and provide a more effective care to these patients.

CONFLICT OF INTEREST No potencial conflict of interest to report.

TITLE CD200 EXPRESSION IN MANTLE CELL LYMPHOMA: A CASE REPORT **CODE** H.14

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ABSTRACT **Introduction:** CD200 (OX-2 antigen), a type I immunoglobulin superfamily membrane protein, has been suggested as an important marker to distinguish mantle cell lymphoma (MCL) from chronic lymphocytic leukemia (CLL) in the study of CD5+ B-lymphocytosis. This marker is uniformly positive in CLL, and is usually absent in MCL. Although uncommon, CD200 may be expressed by a subgroup of MCL, with pathological and clinical features characteristic of the leukemic non-nodal MCL (LnNMCL). This MCL variant, approximately 3% of all MCL, commonly presents with peripheral blood, bone marrow and sometimes splenic involvement but without significant adenopathy (typically defined as peripheral lymph nodes <1-2 cm).

Case report: A 39-year-old man, with no relevant medical history, was admitted to the hospital with night sweats and dry cough. The physical examination was unremarkable. The complete blood count showed leukocytosis (total leukocyte count $29{,}50x10^9$/L) with lymphocytosis (absolute lymphocyte count $26{,}55x10^9$/L). An abdominal ultrasound revealed splenomegaly and periceliac and hepatic hilar adenomegalies, the most significant of which had 1 cm in size. The positron emission tomography scan evaluation showed splenomegaly. Peripheral blood immunophenotypic characterization by flow cytometry revealed 66% pathologic B lymphocytes CD5+ CD10- CD19+ CD20+ CD23- CD79b++ CD200+ HLADR+ IgM++, with lambda light chain restriction, compatible with CD200+ mantle cell NHL-B. Histological examination of the bone marrow showed hypercellularity, with involvement by lymphoid neoplasia of small B lymphocytes. Karyotype analysis and fluorescence in situ hybridization of peripheral blood revealed translocation t(11;14)(q13;q32), resulting in the IGH-CCND1 fusion, in 78% of the cells in the analyzed sample. The MCL International Prognostic Index was determined to be 3, a low risk score. In view of these data, LnNMCL was assumed. The patient remains under clinical surveillance during 15 months, with no clinical symptoms.

Conclusion: CD200 expression identifies a subset of MCL patients in whom SOX11 is frequently negative, likely associated with V(H) hypermutations. They often correspond to LnNMCL cases. This MCL variant is generally associated with indolent disease course. Unlike nodal MCL, these patients have a stable genome and few epigenetic modifications. Clinically, it is similar to CLL and may exhibit aberrant immunophenotype, such as CD200 expression. Patients with this CD200+ variant have shown a better prognosis than those with classic MCL, and frequently can defer initial therapy, without adverse impact on survival. These immunophenotypic changes in MCL could lead to misdiagnosis. Recognizing this variability is important for the diagnostic workup of this lymphoma.

CONFLICT OF INTEREST No potencial conflict of interest to report.

TITLE NEW FLOW-CYTOMETRY APPROACHES FOR THE IN-DEPTH DISSECTION OF TUMOR CELLS IN T-CELL CHRONIC LYMPHOPROLIFERATIVE DISORDERS **CODE** H.15

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ABSTRACT **Introduction:** T-cell chronic lymphoproliferative disorders (T-CLPD) are a heterogenous group of mature T-cell-derived tumors, which classification is difficult and poorly reproducible. Comprehensive immunophenotyping of T-CLPD cells through in-depth analysis of functional- and maturation-associated markers have not been performed. Our aim was to phenotypically compare tumor cells vs normal T-cell subsets, to approach to T-CLPD normal cell counterparts.

Methods: Peripheral blood samples from 30 T-CLPD and 53 age-matched healthy donors were phenotyped and analyzed by flow cytometry, using the 14-color EuroFlow-Immunomonitoring TCD4 tube (and the TCD8 tube for T-LGLL), following EuroFlow SOPs. Patients were classified (WHO2017) as follows: 11 T-PLL; 12 T-LGLL (8 CD8+ 3 CD4+ and 1 Tgd-LGLL) and 7 SS. All samples were analyzed using the INFINICYT software and tools.

Results: In all categories, tumor cells overlapped with the phenotype of conventional (helper or cytotoxic) T cells, i.e. neither regulatory or follicular helper T cells. T-PLL cells showed a heterogeneous chemokine receptor (CR) pattern -except for CCR10, negative in all cases-; despite this, T-PLL cases could be grouped into 3 profiles of CR expression: i) in 5 cases (45%) most tumor cells (≥50%) did not express any CR and cells only partially expressed CD194, CD196 or CD183 (consistent with a naïve CD4T-cell with early maturation to Th2, Th17 or Th1); ii) in 4 cases (36%) ≥90% tumor cells expressed CD194 and/or CD196, i.e. a Th2 or Th17 phenotype; iii) in 2 cases (18%) expressed a non-classical Th profile (CD183+CD194+CD196+ or only CD196+). Regarding their maturation stage, all cases but 3 showed a pattern of central/transitional memory (CM/TM) CD4T-cells (CD27+CD62L+/loCD45RA-); the remaining 3 cases showed a naïve-like cell profile (CD27+CD62L+CD45RA+). SS cells usually expressed high levels of CD194. A Th2 phenotype was represented in 3 cases (43%), together with a non-classical Th phenotype in other 3 cases (43%) and a typical Th17 profile in 1 case (14%). As regards the maturation stage, SS showed a predominantly CM/TM and naïve-like phenotype; however, cells belonging to different maturation stages coexisted in 6 cases (86%). T-LGLL cells (regardless of the T-cell lineage) were CD183+/lo and other CRs- (i.e. Th1) with an effector memory or terminal effector (CD27-CD28-CD45RAlo/+CD62L-/lo) cytotoxic (granzyme B+) phenotype.

Conclusion: T-PLL and SS cells are phenotypically heterogeneous, as reflected by intra- and inter-patient variability, while T-LGLL cells show a homogeneous profile (Th1 effector cytotoxic cell). Despite this, common patterns were found for T-PLL and SS, consistent with a predominant CD183-/lo/CD196-/loCD194-/lo naïve/CM and a Th2/Th17 CM/TM or naïve-like CD4T-cell, respectively. These data support that classification of T-CLPD according to functional and maturational normal T-cell subsets would be useful to gain insight into the biological derivation of T-CLPD.

CONFLICT OF INTEREST No potencial conflict of interest to report.

TITLE IN VITRO ACTIVATION OF WHOLE BLOOD PLATELETS AND ANTIMICROBIAL ACTIVITY OF LEUCOCYTE-PLATELET RICH FIBRIN (L-PRF): A PRELIMINARY STUDY **CODE** H.16

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ABSTRACT **Introduction:** Leukocyte and platelet rich fibrin (L-PRF) is one of the platelet concentrates used to support regeneration and healing process. Many studies showed possible immunological and antibacterial properties of L-PRF. We perform an in vitro study using L-PRF components to analyze its effect on platelet activation, platelet-leucocytes interactions and antimicrobial activity, important components in the healing process.

Methods: L-PRF exudate was used for the in vitro analysis of molecular biomarkers in autologous whole blood by means of flow cytometry. L-PRF membrane was used to evaluate antimicrobial activity using Enterococcus faecalis (ATCC 29212), Pseudomonas aeruginosa (ATCC 27853) and Candida albicans (ATCC 90028).

Results: Our experimental design allowed to evaluate platelet activation and analyze molecular biomarkers of other immune cells and platelet-leukocyte interactions. The results obtained showed that L-PRF can be a valuable tool in healing process, it was efficient in activating platelets of whole blood and inhibiting microbial growth.

Conclusion: From our results, we can conclude that the use of L-PRF exudate, compared with L-PRF membrane, presents some advantages that have to be considered in clinical trials. Additional research on the characterization and quantification of cells and its products present in the L-PRF exudate, as well as on the temporal factor released, are needed.

CONFLICT OF INTEREST No potencial conflict of interest to report.

TITLE THE FLOW CYTOMETRY ANALYSIS PLAYS A RELEVANT ROLE IN BONE MARROW ASSESSMENT AT DIFFUSE LARGE B-CELL LYMPHOMA NOS DIAGNOSIS **CODE** H.17

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ABSTRACT

Introduction: Bone marrow (BM) examination is essential in diffuse large B-cell lymphoma (DLBCL) staging and has a prognostic value. Last recommendations say that PET is the standard technique for evaluating the BM involvement, whereas histology (BMB) is only needed in some cases. The role of flow cytometry (FCM) in this setting has been scarcely evaluated, and it is reported that cases with less than 2% of infiltration by FCM and negative histology should be considered as normal BMs. Our aim was to analyse the FCM utility in detecting BM involvement at DLBCL diagnosis and evaluate its prognostic value.

Methods: Retrospective two-center study of de novo DLBCL NOS cases (2014-2020 period) with complete BM assessment (PET, BMB and FCM) at diagnosis. FCM methodology: 8-colour instruments (Canto II) since 2016, LoQ 0.01%. Clinical and biological variables were compared by descriptive statistics tests, focusing on cases with BM involvement by FCM (FCM+). Survival outcome was evaluated by log rank test and Cox regression model.

Results: Ninety-nine patients were included, 16/99 with BM involvement by FCM (any quantity). The groups FCM+ and FCM- presented similar baseline characteristics (males 69% vs 52%, p=0.2; median age 66 vs 67, p=0.8; cell-of-origin non-germinal center B 62% vs 42%, p=0.1; ECOG ≥2 6% vs 14%, p=0.7) except for three variables: median LDH level (U/l) (380 vs 240, p=0.04), extranodal sites ≥2 (50% vs 20%, p=0.03), and Ann Arbor stage III-IV (94% vs 49%, p=0.001). Of the FCM+ patients, 4/16 (25%) presented BM involvement by a concordant large-cell lymphoma, 11/16 (69%) by a discordant low-grade B-cell lymphoproliferative disorder, and 1/16 (6%) by both large and small B-cell disorders. Median infiltration by FCM was 0.9% (0.05-27), <2% in 10/16 cases. If BM involvement would have been considered as positive in all cases 4/16 would be upstaged. Of the patients with FCM+, 7/16 and 9/16 presented negative BM infiltration by BMB and PET, respectively. With a median follow-up of 29 months, the event-free survival (EFS) after 2 years was 73%, 48% and 0% for FCM-, FCM+ <2% and FCM+ ≥2% groups, respectively (p=0.003); univariative (UV) hazard ratio (HR) for FCM+ <2% vs FCM- was 2.0 (95% CI 0.8-5.2) and for FCM+ ≥2% vs FCM- was 4.6 (95% CI 1.7-12.2). Two-year overall survival (OS) was 77%, 55% and 33% for FCM-, FCM+ <2% and FCM+ ≥2% groups, respectively (p=0.001); UV HR for FCM+ <2% vs FCM- was 1.9 (95% CI 0.7-5.6) and for FCM+ ≥2% vs FCM- was 5.7 (95% CI 2.1-15.7).

Conclusion: Although current guidelines do not include FCM as a relevant analysis in detecting BM involvement at DLBCL diagnosis, it may have an important role specially in cases with discordant infiltration of a low-grade B-cell disorder. Furthermore, BM infiltration by FCM was related with a worse prognosis, without considering BMB or PET. The minimal BM involvement detected by FCM should be considered as extranodal involvement and may have a prognostic implication.

CONFLICT OF INTEREST No potencial conflict of interest to report.

TITLE UNSUPERVISED COMPARISON OF A 15-COLOR PANEL BETWEEN A 4L 20D CONVENTIONAL CYTOMETER VS A 5L 67-D SPECTRAL CYTOMETER WITH THE SAME PIPELINE ANALYSIS IN PKD MUTANT VS WT KNOWN SAMPLES **CODE** H.18

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ABSTRACT

Introduction: With the appearance of spectral flow cytometry and the equipment and algorithms developed for that, the adjustment of the fluorochrome spillover has turned into the analysis of specific spectra and the unmixing of them in multiple labelling populations. This new strategy has allowed the development of panels that can go up to 40 different parameters added in the same tube. Here, we have proposed a direct evaluation of the impact of this new technological approach into the same well known samples by applying the same pipeline of transformation/clustering analysis.

Methods: We have designed a 15 color 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, CD41, CD71, CD117(c-Kit), TER-119, CD135, CD150, F4/80 antigen and viability marker. The samples were acquired both in an 4L BD LSRFortessa X-20™ with a total of 20 detectors and an 5L Cytek® Aurora spectral cytometer with a total of 67 detectors. Both transformation (t-SNE, Flowjo™) of data and clustering (X-shift, Flowjo™) analysis were performed using the same bioinformatics pipeline. Files were also analyzed following conventional procedures. As a validation control, hematopoietic cells from wild type mice and from Pyruvate Kinase Deficient (PKD) mice, suffering of chronic hemolytic anemia and disbalanced erythropoiesis, were analyzed in parallel.

Results: Conventional analysis showed better resolution in data obtained in Aurora in some parameters, such as PE. Using the same pipeline, after transformation and clustering, 35 clusters were detected within Aurora files and 43 in Fortessa files. 14 of 35 (40%) were statistically different between PKD and WT samples in Aurora data and 22 of 43 (51%) in Fortessa. Individual analysis of each cluster showed 14% of segmented ones in case of Aurora vs 40% in Fortessa files. Size of the clusters were no statistically different. In both data sets, populations involved in Erythroid differentiation were statistically different between PKD and WT samples. Importantly, clustering based analysis detected unknown differences in other lineages, including specific T-cell subpopulations

Conclusions: Comparing technological platforms, we have observed a positive impact of the spectral technology, using the same panel applied into well-known samples and applying the same pipeline analysis. In both data sets apart from the expected differences in erythroid populations, automated cell-subset clustering highlighted unknown differences between PKD and WT reinforcing the important relevance of applying unsupervised analyses.

CONFLICT OF INTEREST No potencial conflict of interest to report.

TITLE PERIPHERAL T.CELL LYMPHOMA OF FOLICULAR HELPER T-CELL TYPE: A CASE REPORT **CODE** H.19

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ABSTRACT

Introduction: Tumors with a T follicular helper (TFH) cell phenotype, as defined by the expression of at least two of the following markers: CD10, BCL6, PD1 (CD279), CXCL13, CXCR5 (CD185), ICOS (CD278) and SAP, are excluded from the Peripheral T-cell lymphoma NOS (PTCL) classification. These lymphomas (TFH) are thought to constitute the neoplastic counterpart of TFH cells. According to the WHO classification, the Angioimmunoblastic, Follicular and Nodal Peripheral T-cell lymphomas with TFH phenotype are included in this category (1). In these PTCL with TFH phenotype, neoplastic T cells express most pan-T-cell antigens (CD3, CD2, and CD5) and in most cases are positive for CD4. Surface CD3 may be reduced or absent by flow cytometry (FC)(3). Beside the presence of atypical T-cell population CD3+CD10+, the literature on FC mentions another atypical phenotype, CD3-/+dimCD4+(2-4). It seems a useful feature in distinguishing PTCL of follicular type from morphologic mimics such as reactive hyperplasia or Hodgkin Lymphoma (2-4). Here we present a clinical case of a PTCL with TFH phenotype evaluated by FC with a population CD3- CD4+.

Methods: A 73 years old male with 2 cervical and multiple mesenteric adenomegalies, a slightly splenomegaly, EBV+ was admitted at our institution on January 2021, with a suspicion of lymphoproliferative disease. It was detected a monoclonal peak IgGk. Initially, a lymph node (LN) sample was sent to our laboratory with a suspicion of lymphoproliferative disease and, three weeks later, a bone marrow (BM) sample was received. The analysis of these samples was performed by multiparametric 8 color FC. First, on the LN, a screening tube was performed, to detect the pathologic population and then a panel to characterize this population.

Results: The immunophenotipyc(IF) study of the LN revealed 47.83% of T cells with a normal CD4:CD8 and normal expression of the PAN T-cell markers. Besides this, 6.80% T lymphocytes CD3-CD4+ were present. The IF of this pathologic population was: CD2+CD3-CD4+CD5+dim/+CD7+/++CD8-CD10+dimCD45++. It wasn't possible to complete the immunophenotipic study due to reduced sample volume. The BM sample presented 0.07% of pathological T-cells with an IF: CD2+CD3-cCD3++CD4+CD5+dim/+CD7+/++CD8-CD10+dimCD45++CD279+. The % of these cells could be higher because of bone marrow sample contained several clots. Anatomopathologic analysis of the LN revealed findings compatible with PTCL with TFH phenotype. In BM no malignant T cells were detected.

Conclusion: The immunophenotypics characterization of BM and LN samples revealed the presence of CD3-CD4+ T-cell population described in PTCL of TFH derived cases (3). This aberrant phenotype detection is important because CD10 expression, although characteristic of PTCL with TFH, may be expressed by normal T cells, like a subset of normal TFH and T-cells in LN with reactive follicular hyperplasia (2-4). FC may provide an important clue for complementary diagnostic studies.

CONFLICT OF INTEREST No potencial conflict of interest to report.

TITLE SEX AS A MODERATOR OF THE BLOOD IMMUNE CELLS PROGRESSION DURING AGING: A MOUSE LONGITUDINAL STUDY **CODE** I.01

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ABSTRACT

Mouse models have been widely used as surrogates to understand the physiology of aging, including that of the immune system. While sexual dimorphism in humans has been broadly characterized, the impact of sex in the immune system of animal models, particularly during aging, is poorly studied. Here we longitudinally evaluate male and female Balb/c mice, from 3 to 18 months old, with respect to the main blood populations of both innate and adaptive immune systems, assessed by flow cytometry. To evaluate the cell composition trajectories during aging, linear mixed models were used. In general, the percentage of neutrophils, monocytes, eosinophils and natural killer (NK) cells Ly6C- increase with aging; while that of NK cells Ly6C+, B and T cells (including CD4+ and CD8+ subsets) decrease. Interestingly, males present higher percentages of neutrophils and activated monocytes Ly6Chigh. In contrast, females present higher percentages of total T cells, both CD4+ and CD8+ T cells, as well as of eosinophils and NK cells. Both males and females display similar percentages of B cells, even though with different accelerated progressions over time.

Altogether this study revealed an overall decrease in the adaptive immune cells and an increase in innate cells and an age-related sexual dimorphism in the proportion of immune cells in circulation, resembling some features of the human immune system aging. These observations further strengthen the need to control for the impact of sex when addressing the immune system aging using animal models.

CONFLICT OF INTEREST No potencial conflict of interest to report.

TITLE HIV-INFECTED PATIENTS UNDERGOING ANTIRETROVIRAL THERAPY HAVE PERSISTENTLY REDUCED NUMBERS OF NATURAL KILLER CELLS WITH AN ALTERED PHENOTYPE **CODE** I.02

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ABSTRACT **Introduction:** HIV infection causes targeted deletion of CD4+ T cells and reduced percentages of other immune cells, including Natural Killer (NK) cells. If left untreated, HIV infection results in severe immunodeficiency and death. Antiretroviral therapy (ART) leads, for most of the patients, to the recovery of CD4+ T cells counts. However, individuals on long-term ART with good CD4+T cells immune recovery still present increased susceptibility to opportunistic infections and higher incidence rates of cancer, when compared to the overall population. Little is known on the impact of ART on the homeostasis and phenotype of NK cells. Given the importance of NK cells as a first line of defense against certain viral infections and malignant cells, we hypothesize that increased susceptibility to infection and malignancies may be linked to poor NK cell recovery, in terms of numbers and/or phenotype. Therefore, we aimed to evaluate the alterations of NK cells from ART initiation and after the first 36 months (M) of therapy, in comparison to healthy control (HC) individuals.

Methods: We used a cohort of HIV-infected patients followed since ART initiation up to 36M to evaluate CD56+CD3- NK cells, and their expression of several activating and inhibitory surface receptors, in cryopreserved peripheral blood mononuclear cells (PBMCs).

Results: We observed that the percentages and total numbers of NK cells in HIV-infected individuals are persistently lower than in HC, irrespectively of being on ART. In addition to the lower number, NK cells revealed a persistent distinct phenotype, characterized by decreased percentages of cells expressing the activating Nkp30 and the inhibitory receptor NKG2A. By cluster analysis we observed that, at 36M of ART, HIV-infected patients with lower percentages of CD56dim NK cells expressing NKG2A also presented lower percentages of cells expressing the activating receptors NKp30 and NKp46. Regarding NK cells maturation profile, we observed higher percentages of CD57+ cells, particularly the CD57+NKG2A- sub-population, in HIV-infected patients, namely at 36M of ART, in comparison to HC.

Conclusion: Our observations suggest that there is an accumulation of terminally differentiated NK cells, described to have poor potential to respond to new stimuli, in HIV infected individuals under ART. These results may elucidate the role of NK cells dysfunction in the development of clinical complications in ART treated HIV-infected patients and open new avenues for the discovery of new therapeutics targeting NK cells, hence contributing to improved quality of life in HIV-infected individuals.
Keywords: Human immunodeficiency virus (HIV) infection, antiretroviral therapy (ART), natural killer (NK) cells, immune recovery.

CONFLICT OF INTEREST No potencial conflict of interest to report.

TITLE IMMUNOLOGICAL BIOMARKERS OF FATAL COVID-19: A STUDY OF 513 PATIENTS **CODE** I.03

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ABSTRACT **Introduction:** Information on the immunopathobiology of coronavirus disease 2019 (COVID-19) is rapidly increasing. However, most studies analyzed relatively small series of patients and the identification of immune features predictive of fatal outcome remains an unmet need. Our aim was to characterize immune responses to severe acute respiratory syndrome coronavirus (SARS-CoV-2) infection in a large cohort of patients to identify high-risk immune biomarkers.

Methods: Multidimensional flow cytometry was used to conduct holistic and unbiased analyses of 17 immune cell types on 780 peripheral blood samples obtained from 513 COVID-19 patients and from 24 patients with non-SARS-CoV-2 infections as well as 36 healthy adults. 167 COVID-19 patients had 207 longitudinal samples collected over time. RNA sequencing on FACSorted cells and high-resolution flow cytometry were used to perform deeper characterization of various myeloid and lymphoid subsets in 14 patients and 4 healthy adults.

Results: Immune profiles of COVID-19 patients were generally similar to those of age-matched patients with non-SARS-CoV-2 infections, but significantly different from those of age-matched healthy adults. When compared to the later, COVID-19 patients showed increased percentages of neutrophils, CD4+CD56+ T-cells, and circulating plasma cells (PC), whereas levels of basophils, eosinophils, and non-classical monocytes, as well as double-negative, CD8loCD56-, CD8-/loCD56+, and CD8hiCD56- T-cells, and B-cells were decreased. Both transcriptional and immunophenotypic data in myeloid and lymphoid subsets suggested an association between COVID-19 severity and neutrophil activation, as well as significantly reduced levels of specific adaptive immune cell types. Unsupervised clustering analysis of 513 patients revealed three immunotypes in response to SARS-CoV-2 infection. One of them, present in 14% of patients (n=74), was characterized by significantly lower percentages of all immune cell types except neutrophils and circulating PC, and was significantly associated with more severe disease. On multivariate analysis incorporating age and comorbidities, the frequency of B-cells and non-classical monocytes were independent prognostic factors for survival. Indeed, <1% B-cells in peripheral blood was most strongly associated with risk of death. Among patients with immune monitoring during follow-up, significant changes in the relative distribution of eight immune cell types, including basophils, CD8loCD56- T-cells, and B-cells, were observed from the first to last peripheral blood sample between patients who survived or died.

Conclusion: Our results highlight the importance of B-cells in response to SARS-CoV-2. Reduced percentages of B-cells and non-classical monocytes are high-risk immune biomarkers that could be readily implemented in routine practice for risk-stratification of COVID-19 patients.

CONFLICT OF INTEREST No potencial conflict of interest to report.

TITLE ROLE OF FLOW CYTOMETRY IN THE MONOCYTES/MACROPHAGES-ASSOCIATED PATHOLOGICAL INFLAMMATION IN PATIENTS WITH SARS-COV-2 INDUCED INTERSTITIAL PNEUMONIA **CODE** I.04

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ABSTRACT **Background:** The SARS-CoV-2-induced complications have been associated with the most vulnerable populations. An unbalanced inflammatory response define the pathological hallmark underlying severe presentations of COVID-19. However, the complex immune cell interplay in COVID-19 remain a challenge.

Methods: We sought to identify potential associations between immunological monocyte-macrophage profile in 15 previously healthy patients younger than 50 years with SARS-CoV-2 positive bilateral interstitial pneumonia and 15 patients with SARS-CoV-2 positive bilateral interstitial pneumonia with a history of solid malignancy admitted to the Hospital Clínico San Carlos, Madrid, Spain.

Results: Five of 15 patients previously healthy patients with SARS-CoV-2 positive bilateral interstitial pneumonia presented severe respiratory failure with the need for assisted ventilation. These patients showed an increase in the percentage of intermediate monocytes when studying the phenotype of peripheral monocyte subset compared to those who did not require ventilatory assistance. An increase of intermediate monocytes population was observed in oncologic patients and SARS-CoV-2 positive bilateral interstitial pneumonia, without a clear association with severe respiratory failure. This finding could be derived from a previous dysregulated cytotoxicity state in these patients.

Conclusion: The proinflammatory monocytes behave as mediators of the hyper-inflammatory response produced by SARS-CoV-2. Therefore, the study of monocyte-macrophage differentiation through flow cytometry could be a predictive tool in the development of severe cases.

Our gratitude to CRIS-Cancer Foundation.

CONFLICT OF INTEREST No potencial conflict of interest to report.

TITLE STIMULATION WITH MYCOBACTERIAL GLYCOLIPIDS AND PPD REVEALS DIFFERENT INNATE IMMUNE RESPONSE PROFILES IN ACTIVE AND LATENT TUBERCULOSIS **CODE** I.05

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ABSTRACT **Introduction:** Upon infection with Mycobacterium tuberculosis (Mtb), the immune response might clear the bacteria, control its growth leading to latent tuberculosis (LTB), or fail to control Mtb resulting in active tuberculosis (ATB). There is however no clear understanding of the features underlying a more or less effective response. Research on the host response to Mtb has been mainly focused on antigens of protein/peptide nature although the immune response is initiated mainly through the interaction of Mtb cell envelope components, mostly glycolipids, with cells of the innate immune system. Lipoarabinomannan (LAM) and its precursors the phosphatidyl-inositol mannosides (PIM2, and PIM6) are abundant in the bacterial cell envelope and interact with both innate and adaptive immune cells. It is known that both LAM and PIM have immunomodulatory properties, but the patterns of response to glycolipids are still underexplored.

Methods: We performed a detailed assessment and simultaneous comparison of the immune response to PIM and LAM from Mtb in peripheral blood mononuclear cells (PBMCs) in individuals with active or latent TB and compared with healthy controls (HC). For that, we used in-house produced PIM and LAM and dissected the immune profiling by mass cytometry measuring simultaneous 37 cellular markers at the single-cell level to allow high-resolution of cellular composition. Mass cytometry data was analyzed using the R-package Cytofkit v1.12.0, which includes an integrated pipeline for mass cytometry analysis.

Results: Stimulation with the Mtb glycolipids LAM and PIM led to the early production of several different cytokines in multiple cell subsets. However, individuals with ATB or LTB responded with less cytokine production by myeloid cells and somewhat by B and T cells upon stimulation with Mtb antigens. PIM induced a polyfunctional immune response, mainly in antigen-presenting cells, leading to the production of both proinflammatory (IL-2, IL-6, IL-17A, TNF-alpha and GM-CSF), and IL-4 and IL-10 cytokines, but not IFN-γ. LAM triggered weaker but similar responses. Expansion of myeloid subsets producing cytokines in response to PIM and LAM was reduced in ATB and LTB compared to HC, but more in LTB, suggesting a hyporesponsive/tolerance pattern.

Conclusion: The effect of PIM and LAM on myeloid cells in individuals with ATB and LTB suggests a pathogen-specific innate immune response that requires further exploration. By defining the mechanistic underlying the response to Mtb glycolipids, such as PIM, new vaccine strategies and correlates of protection may be developed.

CONFLICT OF INTEREST No potencial conflict of interest to report.

TITLE HIGHER NAÏVE TO MEMORY T CELLS AND ALTERED REGULATORY CELLS CHARACTERIZE MULTIPLE SCLEROSIS AT CLINICAL ONSET **CODE** I.06

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ABSTRACT **Introduction:** The immune system has the tricky task of fighting pathogens, while being tolerant to self-peptides avoiding autoimmune diseases. Failure at maintaining tolerance, particularly at the level of T cells, which are produced in the thymus, is described to underlie multiple sclerosis (MS) pathogenesis. MS can manifest in different forms, being the most common (~85%) the relapse-remitting MS (RRMS), characterized by bouts of disability followed by recovery. It has been suggested that RRMS patients have lower thymic export, an aged adaptive immune system and that the regulatory capacity of regulatory cells is reduced comparing to healthy controls (HC). However, it is still not clear if those alterations are already present at disease onset. Our aim is to study the immune system of disease modifying drugs' naïve RRMS patients at disease onset regarding thymic export, naïve and memory T cell subsets and regulatory cells phenotype.

Methods: Newly diagnosed RRMS patients (n=27) and sex and age-matched HC (n=35) were recruited. Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood by gradient centrifugation, enumerated and cryopreserved until use.

Results: In comparison to HC, newly diagnosed RRMS patients presented lower percentages, but similar numbers, of CD4+ recent thymic emigrants (CD31+CD45RA+CCR7+CD45RO-). In accordance, no differences were observed on sjTREC levels. Newly diagnosed RRMS patients had higher percentages of naïve (CD45RA+CCR7+CD45RO-) CD4+ T cells, and lower percentages of total memory cells (combination of central memory [CD45RA-CCR7+CD45RO+], effector memory [CD45RA-CCR7-CD45RO+] and terminally differentiated [CD45RA+CCR7-CD45RO-]) for both CD4+ and CD8+ T cells. The numbers of memory CD4+ T cells were lower in newly diagnosed RRMS patients, as a result of lower numbers of central memory cells. Altogether, these alterations translated into a higher naïve/memory ratio of CD4+ T cells on RRMS patients. Compared to HC, RRMS patients had higher numbers of naïve and lower of activated HLA-DR+ (most suppressive) Tregs [CD127-CD25+FOXP3+CD4+ T cells]. No major alterations were observed on the percentages and numbers of natural killer (NK; CD3-CD56+) and NK T-like (CD3+CD56+) cells, and their subsets. Notwithstanding, RRMS had higher percentages of immature NK cells expressing the inhibitory receptors KLRG1 and NKG2A, and lower percentages of NK T-like cells expressing the inhibitory receptors KIR2DL2/3 and KIR3DL1.

Conclusion: Our results suggest that RRMS patients at disease onset and HC have similar thymic export of T cells, but the former display higher naïve/memory ratio of CD4+ T cells, signs of reduced Treg suppressive capacity and higher NK but lower NK T-like cells proneness to be inhibited. Functional studies will be essential to further understand the mechanisms underlying these alterations and their impact on disease manifestation.

CONFLICT OF INTEREST No potencial conflict of interest to report.

TITLE FLOW CYTOMETRY IN BLOOD CLASSIFICATION OF MYCOSIS FUNGOIDES/SÉZARY SYNDROME **CODE** I.07

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ABSTRACT **Introduction:** Mycosis fungoides (MF) and Sézary syndrome (SS) are the classic types of Cutaneous T-cell lymphomas. The assessment for Sézary cells in the peripheral blood is of the utmost importance since its presence has prognostic significance, with added relevance regarding management and therapy response. Hence, the need to establish a universal and objective definition for the blood classification in staging MF/SS. We aim to apply the EORTC 2018 criteria (using Flow cytometry) for blood classification in patients with MF/SS, assess the prognostic role of B1 stage and compare the flow cytometry results with the molecular study of T-cell receptor clonality.

Methods: We used the EuroFlow immunophenotyping protocol (Tube 1 T-cell chronic lymphoproliferative syndromes: CD4-V450/CD45-V500/CD7-FITC/CD26-PE/CD3-PerCPCy5.5/CD2-PECy7/CD28-APC/CD8-APCH7). We took fresh peripheral blood samples from 10 normal individuals, 17 patients with non-neoplastic erythroderma, 26 patients diagnosed with MF and 1 patient diagnosed with SS. None of the patients had received systemic therapy prior to the study. All samples were analysed using Infinicyt software.

Results: We detected CD4+ T-cells with loss of CD26 and/or CD7 in both normal individuals and in cases of reactive erythroderma, even as much as to classify 47% of reactive erythroderma as stage B1. Meanwhile, 73% of MF were classified as stage B1 and the only Sézary syndrome patient was classified as stage B2. The clinical-biological features of 27 patients with MF/SS are shown in table 1. The presence of T-cell clonality in peripheral blood was assessed in 18 of 27 patients with MF/SS diagnosis: it was found in 16% of the stage B0 patients and in 36% of stage B1 patients, although its prognostic impact remains unknown. Table 1. Stage according to CD4+/CD7- and/or CD4+/CD26- T-cell count REACTIVE ERYTHRODERMA (n=17) MF/ SS (n=27) B0 (1000/mm3) 0 (0%) 1 (3,7%)

Conclusion:
- Blood staging requires standardising of technology to obtain reproducible results, especially due to the absence of a specific marker for Sézary cells.
- Further studies are required in order to assess the prognostic impact of a low blood involvement (stage B1) in MF patients.
- The B0 and B1 stages should be subdivided according to the presence/absence of T-cell clonality in peripheral blood, in order to assess its prognostic significance.

CONFLICT OF INTEREST No potencial conflict of interest to report.

TITLE CD45RA, CD8β, AND IFNγ REPRESENT A NOVEL IMMUNE SIGNATURE OF HUMAN COGNITIVE FUNCTION **CODE** I.08

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ABSTRACT

Introduction: For decades, the immunological system (IS) and the central nervous system (CNS) were considered two separated and independent systems. It is now well-established that the IS and CNS are in continuous crosstalk, though their interaction is far from being understood. A possible role for the IS in maintaining CNS homeostasis has long been a matter of debate, although the cells of the IS have long been considered detrimental in the context of neurodegenerative diseases. Noteworthy, recent studies have contributed to the view that in humans the adaptive immunological system can influence cognitive functions of the brain.

Methods: We have undertaken a comprehensive immunological analysis of lymphocyte and monocyte populations in a cohort of elderly volunteers (age range, 64–101) differing in their cognitive status. Peripheral blood mononuclear cells were isolated from blood samples collected from elderly volunteers and were immediately phenotyped and functionally characterized by flow cytometry. Cytomegalovirus (CMV) seroprevalance was evaluated by ELISA in plasma samples from the elderly volunteers.

Results: Hereby, we report on the identification of a novel signature in cognitively impaired elderly characterized by: (1) elevated percentages of CD8+ T effector-memory cells expressing high levels of the CD45RA phosphate receptor (TEMRAhi); (2) high percentages of CD8+ T cells expressing high levels of the CD8β chain (CD8βhi); (3) augmented production of IFNγ by in vitro activated CD4+ T cells. Noteworthy, CD3+CD8+ TEMRAhi and CD3+CD8βhi cells were associated with impaired cognition. CMV seroprevalence showed that all volunteers studied but one were CMV positive.

Conclusion: To our knowledge, this is the first proof in humans linking the amount of cell surface CD45RA and CD8β chain expressed by CD8+ TEMRA cells, and the amount of IFNγ produced by in vitro activated CD4+ T cells, with impaired cognitive function in the elderly.

CONFLICT OF INTEREST No potencial conflict of interest to report.

TITLE THE USE OF FLOW CYTOMETRY FOR THE CHARACTERIZATION OF ENDOMETRIAL IMMUNE CELLS IN WOMEN WITH IDIOPATHIC RECURRENT PREGNANCY LOSS **CODE** I.09

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ABSTRACT

Introduction: The endometrial immune environment, rich in NK cells, has an important role for embryo implantation and pregnancy´s maintenance. Women suffering from recurrent pregnancy loss (RPL) have abnormal local immune profiles, but data available rely mostly in immunohistochemical techniques. Recognizing the need for a more detailed characterization of uterine lymphocytes, we aimed to develop a flow cytometry strategy to analyse uterine biopsies from women with idiopathic RPL (iRPL).

Methods: Uterine biopsies were collected during the mid-luteal phase (7-10 days after LH peak) from Women with iRPL (n=12), using the Pipelle® system. All biopsies were disaggregated in the MediMechine II for 60s, using BD Medicon chambers (50 µm) and Filcon filters (50 µm). Finally, cell pellets were resuspended in 1mL of BD FACS Flow. A single platform strategy with BD TrucountTM tubes was applied to obtain absolute counts, considered as cells/µL per 1cm of Pipelle®. The following mAbs were used to characterize NK and T-cells: CD3, CD16, CD45, CD56, CD57, NKG2D, NKp30, NKp44, NKp46, 2B4. All samples were run in duplicates (i.e., 2 portions of the same biopsy run in parallel) and acquired in an 8-colour BD FACS Canto II. Statistical analysis was performed with GraphPad Prism (significance at p-value<0.05).

Results: The samples analysed presented lymphocyte counts between 12,95–145,20 cells/µL per centimetre of Pipelle® biopsy, with a mean coefficient of variation (CV) between replicates of 7,61%. For the percentages of the major immune populations identified (T-cells, CD8+ T-cells, NK and NK CD56bright cells), replicates revealed mean CVs<15%. Altogether, T-cells were the most prevalent immune population (34,75–76,88%), and within these, CD8+ T-cells (39,33–67,81%). However, when considering T-cell subsets separately, NK cells (31,60–69,65%) were the more abundant cells in most samples, as described in literature. Additionally, the characterization of specific markers in distinct subsets presented good reproducibility (mean CVs<14%), particularly PD-1 and 2B4 in CD8+ T-cells, and NKp46 and 2B4 in NK cells. Higher discrepancies were achieved in samples with low cellularity, with less than 500 lymphocytes acquired per tube, which probably would benefit from either live gating acquisition or the use of replicate tubes, to assure the necessary performance.

Conclusion: Our results support flow cytometry as a promising and reproducible technique for a deep characterization, through a minimally invasive way, of endometrial immune cells, with the possibility to establish reference ranges and identify subsequent alterations. In the future, we aim to further implement this assessment in a larger cohort of women (including healthy women and women suffering from diverse obstetric complications), as we believe that this information can help clinicians to follow up women suffering from iRPL and other complications, allowing more individualized approaches for each patient.

CONFLICT OF INTEREST No potencial conflict of interest to report.

TITLE DUODENAL LYMPHOGRAM AS A NECESSARY TOOL IN THE DIFFERENTIAL DIAGNOSIS OF CELIAC DISEASE IN ADULTS **CODE** I.10

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ABSTRACT

Introduction: Celiac disease (CD) is an immune-mediated systemic disorder triggered by gluten intake, which can cause alterations to duodenal mucosa, ranging from intraepithelial lymphocytosis to villous atrophy. The latter lesions are not exclusive of CD and may be present in other digestive disorders. Duodenal lymphogram has been proven to be a useful tool in the diagnosis of CD. The typical finding is an increase of total intraepithelial lymphocytes (IELST), as well as an increase of IELS TCR-γδ+ associated with a decrease of iNK cells. The aim of this study was to describe the characteristics of the duodenal lymphogram in patients with CD and compare them with those found in patients with atrophic gastritis, H. pylori infection and controls (normal findings).

Materials and methods: Duodenal biopsy samples received in our service between December 2019 and December 2020 were included in this study. From the total of 66 samples, only 5 belonged to pediatric patients, and these were excluded from the study. Adult patients were distributed into two cathegories: I) CD and II) non-CD. Additionally, the last were divided into three subgroups: IIa) atrophic gastritis, IIb) H pylori infection and IIc) controls. Based on the established protocol, the study of the duodenal lymphogram was carried out using flow cytometry in intestinal biopsy samples. Cut-off values were: IELST>12%, IELS TcR-γd+>10% and iNK<40%. SPSS Statistics 17.0 was used to perform the statistical analysis. Results were considered significant when $p \leq 0.05$.

Results: Duodenal lymphogram from CD patients was compared with that of non-CD patients. As expected, the results showed significantly higher values in celiac patients for IgA-tTG (p=0.023), IgG-DPG (p=0.022), IELST (p=0.012), IELS TcR-γδ+ (p<0.001) and lower iNK values (p=0.001) than non-celiac patients. When non-CD patients were divided into different groups according to their digestive pathology (gastritis; H pylori infection; and controls), we obtained similar results: patients diagnosed with CD presented significantly higher values of TcR-γδ+ than the rest of the groups. Besides, iNK values were significantly lower in CD compared to patients with atrophic gastritis (p=0.02) or controls (p=0.002), but no significant difference was observed when compared with the H pylori infection group. Finally, according to the Marsh classification, an inverse relationship between iNK levels and the degrees of Marsh scale was observed.

Conclusion: Our results highlight the usefulness of the duodenal lymphogram in the differential diagnosis of CD, especially the combination of TcR-γδ+ and iNK counts. iNK subpopulation may be considered as a marker of intestinal mucosal aggression.

CONFLICT OF INTEREST No potencial conflict of interest to report.

TITLE CELLULAR T SPECIFIC IMMUNE ASSAY FOR THE ASSESSMENT OF S1-MRNA COVID-19 VACCINE (BNT162B2) RESPONSE BY FLOW CYTOMETRY **CODE** I.11

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ABSTRACT

Introduction: With the arrival of Covid-19 vaccines, the assessment of both humoral and T-specific cellular response after the two doses would be interesting in order to clarify the vaccine efficacy. In this study, a method validated by the Spanish Society for Immunology (SSI) based on flow cytometry to assess the SARS-Cov2 specific cellular response has been tested.

Material and methods: A total of 53 volunteers were recruited for the study after given consent at Marqués de Valdecilla University Hospital. All the subjects were immunized with S1-mRNA Covid-19 vaccine (BNT162b2) first dose from 6th to 12th January and the second dose from 28th to 29th January. In order to assess specific cellular response against SARS-CoV2 S protein, 20 days after the first dose and 30 days after the second dose samples were analyzed. The T response assessment was performed by following the SSI agreed protocol (1), and the activation induced markers CD25 and CD134 expression was studied by calculating the stimulation index referred to No Stimulation control. Samples were acquired by CytoFLEX cytometer (Beckman Coulter) after 24 hours of culture.

Results: Twenty days after the first dose, the median ratio after S-peptide activation (S-ratio) was statistically higher than M-ratio and N-ratio: 3.3 (2.0-4.4) vs 1.8 (1.4-3.0) and 1.2 (1.0-1.7), p <0.01 and =3 to consider as positive specific reaction, as previously shown by Grifoni et al. Cell 2020. The specific response against S-protein was confirmed in 48 of 53 volunteers. All of them developed specific S-protein IgG, hence the sensitivity of the assay was 90.5 %.

Conclusion: Despite of inherent limitations of the functional assays, the SSI agreed protocol to assess specific cellular response against SARS-CoV2, showed a sensitivity greater than 90% which confirms its possible role to monitor not only cellular immune response after Covid-19 disease but also in the vaccine-induced response.

Link: https://www.geclid.es/file.php/397/Protocolo_respuesta_celular_AIM_SARS-CoV-2_v6.pdf

CONFLICT OF INTEREST No potencial conflict of interest to report.

TITLE EVALUATION OF EXPRESSION OF HLA-DR IN MONOCYTES BY FLOW CYTOMETRY AS AN INDICATOR OF INFLAMMATORY DISEASE **CODE** I.12

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ABSTRACT **Introduction:** Monocytes play an important role in immune regulation. These cells are responses for antigen presentation to lymphocytes leading to initiation of humoral and cellular immune response. Monocytes presents functions as phagocytose and cytokines production mediated by cellular surface molecules expression. The human main histocompatibility complex (MHC) is the human leukocyte antigen (HLA). HLA-DR is a type of HLA molecule encoded in HLA gene class II region. HLA-DR is expressed on membrane of antigen presenting cells (APC's). HLA-DR molecules are important to presentation of antigens to CD4 + T cells, are mostly expressed in monocytes and reflect activation state of these cells. In inflammatory and infectious diseases occur decrease of HLA-DR molecule expression on monocytes surface. This study aimed investigate the potential use of HLA-DR molecule expressed in monocytes quantify by flow cytometry method as a biomarker to inflammatory disease diagnostic in outpatients and intensive care unit (ICU) patients.

Methods: Peripherical blood samples of ICU patients hospitalized between May and July of 2018 were analyzed using a flow cytometer to quantify HLA-DR molecule expression in monocytes. Demographic and clinical laboratory data were prospectively analyzed. The value of Immature Granulocyte % (IG%) higher than 0.65% was used to describe the outcome cases (inflammatory disease), as previously reported. Cut-off of MFI HLA-DR were compared with others laboratory variables commonly changed in inflammatory disease as White Blood Cells (WBC) count, IG% count, C-Reactive Protein (CRP) levels, band cells percentage in peripherical blood and detection of "flags" by hematological analyzer.

Results: 78 ICU patients were included in this study. ROC analyze revealed that cut-off of HLA-DR mean fluorescence intensity (MFI) to detect the outcome was 1379, with sensitivity of 63.6% and specificity 97.1%. Area Under Curve (AUC) was 0.833 (95% Confidence Interval 0.746-0.921) and p value 10% (p11000 cells/μL (p0.65% (p<0.001) and "flags" detection (p=0.001).

Conclusion: MFI values of HLA-DR below 1379 were associated to other biomarkers of inflammatory disease. Quantify of HLA-DR expression in monocytes by flow cytometry method presents high specificity to detect inflammatory disease in ICU patients.

CONFLICT OF INTEREST No potencial conflict of interest to report.

TITLE COMPLETE CD4 EXPRESSION DEFECT – CASE REPORT **CODE** I.13

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ABSTRACT **Background:** CD4 is a T cell membrane coreceptor involved in thymic selection and T cell activation. Complete multilineage CD4 expression defect due to a CD4 gene mutation is an extremely rare disorder with only one case recently reported in the literature. Distinctively to idiopathic T CD4 lymphocytopenia, which often courses with opportunistic infections, the single reported case of CD4 expression defect presented itself with treatment refractory warts without other recurrent infections or features of disordered immunity.

Case presentation: We present a case of a 52-year-old male, born from consanguineous parents, referred to our hospital with a 4-decade history of exuberant non-pruritic verrucous skin lesions. He was an active smoker with 45 pack-years of smoking load. Prior medical history included pulmonary and laryngeal tuberculosis at ages 10 and 20, respectively; a dental abscess requiring surgical drainage at age 46 and gastrointestinal pathology comprehending hemorrhoids, hiatal hernia and erythematous gastritis. Relevant aspects of his family history comprised a brother recently diagnosed with a complete CD4 expression deficiency who presented with Whipple's disease, and a nephew with dermatological lesions reportedly similar to the patient's. Physical examination disclosed well-circumscribed skin to brown colored verrucous papules involving torso, chest, neck, limbs and genitals suggestive of common warts and genital warts. Relevant laboratory findings included moderate lymphopenia (1053/μL) and reduced IgG levels (586mg/dL). Flow cytometry revealed complete absence of CD4+ T cells (G, c.245C>G). This is the same homozygous mutation detected on his brother, but distinct from the one previously reported in the literature. He started on isotretinoin in July 2020, with regression of the skin lesions except for the genital area. There is no record of new intercurrences to date.

Discussion: We report a new case of multilineage CD4 expression defect. Similar to the previously reported case, this patient presented with extensive verrucous dermatosis. Distinctively, he had a history of mycobacterial infection and B cell lymphopenia with hypogammaglobulinemia. The fact that CD4 deficient patients do not have severe recurrent infections may suggest that the expanded TCR-αβ+ CD4-/CD8- T cells are contributing to maintain a normal immune response, and that the CD4 molecule has a less relevant role supporting T cell development and activation than previously thought.

CONFLICT OF INTEREST No potencial conflict of interest to report.

TITLE DISTINCT IMMUNE SIGNATURE IN cOVID-19 PATIENTS WITH CARDIAC DYSFUNCTION **CODE** I.14

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ABSTRACT **Introduction:** SARS-CoV-2 infection is associated with multiple cardiac manifestations. In severe COVID-19 patients, diagnostic transthoracic echocardiography (TTE) allows early recognition of cardiac injury with an impact on clinical management, reducing organ dysfunction and mortality. This injury may occur by direct viral cytopathic effects or virus-driven immune activation, resulting in heart infiltration by inflammatory cells. We aimed to explore clinical and immune parameters in COVID-19 patients admitted to the intensive care unit (ICU) to identify distinctive features in patients with cardiac injury. Methods: We enrolled 30 patients >18 years with positive SARS-CoV-2 RT-PCR admitted to the ICU. Exclusion criteria comprised acute myocardial infarction and pulmonary embolism. On days D1, D3, and D7 after admission, patients performed TTE with speckle tracking (STE) technology, hemogram, cardiac (pro-BNP; troponin) and inflammatory biomarkers (ESR; ferritin; IL1β; IL6; CRP; d-dimer; fibrinogen; PCT; adrenomedullin, ADM) and immunophenotyping by flow cytometry.

Results: Patient's mean age was 60.7y and 63% were males. Hypertension was the most common risk factor (73%; with 50% of patients under ACEi or ARA), followed by obesity (40%, mean BMI=31kg/m2). Cardiac dysfunction was detected by STE in 73% of patients: 40% left ventricle (LV) systolic dysfunction, 60% LV diastolic dysfunction, 37% right ventricle systolic dysfunction. There were no significant differences between patients with (DYS) and without cardiac dysfunction (nDYS) regarding mortality, hospitalization days, remdesivir use, organ dysfunction, cardiac or serum biomarkers, except for ADM (increased in nDYS patients at D7). Along all timepoints, DYS patients showed persistent low lymphocyte counts (recovered at D7 in nDYS patients). Activated CD4 and CD3 T-cell counts were similar between groups at D1 and D3, but both subsets increased in nDYS patients at D7 (CD4, p=0.005; CD3, p=0.046). Activated CD8 T-cells (%) were also decreased at D3 in DYS patients (p=0.037), recovering afterwards, while nDYS showed similar values in all time points. At admission, effector memory (EM) T-cells were higher in DYS patients (CD4%, p=0.025; CD8%, p=0.035; CD8 counts, p=0.011), who kept increased %EM CD4 T-cells in all time points (p≤0.046). DYS patients had lower platelets at D3, with slower recovery in platelet counts and CRP levels. CRP decreased at D7 in nDYS patients (p=0.009). This evolution was not paralleled by any cardiac function parameter, and patients recovered with increasing P/F ratio (lesser extent in DYS group).

Discussion: Our study shows a differential immune trait in DYS patients at ICU admission, with persistent lymphopenia, enriched EM T-cell subsets, and altered T-cell activation, suggesting distinct inflammatory states or migration patterns in patients that develop cardiac injury. Thus, T-cell dysregulation might undermine immunoprotection and contribute to disease severity.

CONFLICT OF INTEREST No potencial conflict of interest to report.

TITLE CD123 EXPRESSION IN PEDIATRIC ACUTE MYELOID LEUKEMIA **CODE** I.15

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ABSTRACT **Introduction:** Pediatric Acute Myeloid Leukemia (AML) represents around 15% of all pediatric acute leukemias. Survival rates in children are around 70%, but relapse remains the most important cause of failure. The prognosis of AML with FLT3-ITD mutation is generally poor, being associated with a higher risk of relapse. In AML, as well as in other hematological malignancies, the expression of CD123 has gained particular attention. CD123, the α chain of the interleukin-3 receptor, is a cytokine receptor that is normally expressed on plasmacytoid dendritic cells, eosinophils, and neutrophils. Different studies showed an increased frequency of CD123+ cells within the immature cell population from pediatric AML. CD123 expression has also been reported to be correlated with mutations in FLT3 or NPM1.

Methods case report: Results Male, 5 years old, diagnosed with AML in the last quarter of 2020. Motivated by anemia and thrombocytopenia in a routine analytical study, AML was confirmed by bone marrow (BM) immunophenotyping (IF), with 22% of blasts (CD34+d/+, CD4-/+, CD7-/+, CD9-, CD11b-/+, CD13-/+, CD14-, CD15-/+, CD16-, CD19-/+, CD22-, CD33+, CD36-, CD38++, CD56-, CD64-/++, CD117+d/+, CD123+, HLADR+d/+, NG2-, TdT-). BM cytogenetic study revealed the presence of FLT3-ITD mutation. Induction treatment was immediately initiated, and after two cycles, less than 5% of blasts were detected on BM. Consolidation treatment was started, and after one month, the disease relapsed, with 6% of blasts (CD123+) on the peripheral blood, and more than 30% of myeloid blasts on the BM.

Conclusion: Recent advances in cancer pathogenesis allowed the rise in studies of new biomarkers that can have significant diagnostic, prognostic, and therapeutic implications. Minimal residual disease (MRD) is an independent prognostic factor for relapse in pediatric AML. As said before, FLT3-ITD mutation at diagnosis represents an increased relapse risk. However, MRD negativity for this mutation is not a reliable disease marker, since around 25% of patients have FLT3-ITD negative relapses, while positive at diagnosis. With this, IF has gained an important role in MRD detection. Interestingly, recent studies support CD123 as a diagnostic marker of MRD, as it is overexpressed on both leukemic stem cells and more differentiated leukemic blasts and is low on normal hematopoietic stem cells. It has also been reported that CD123 is overexpressed in most AML cases with mutations on FLT3 or NPM1. Most notably, this fact makes it also an important target for the development of new therapies. Tagraxofusp, a CD123 targeting agent recently approved for the treatment of blastic plasmacytoid dendritic cell neoplasm, is currently in clinical trials for AML. The development of a variety of approaches to target CD123 has produced promising results and has led to numerous clinical trials, creating an opportunity to potentially improve rates of long-term remission and survival in AML.

CONFLICT OF INTEREST No potencial conflict of interest to report.

TITLE ALTERATIONS OF LYMPHOCYTE IMMUNOPHENOTYPE AS POSSIBLE PREDICTORS OF SUSCEPTIBILITY TO INFECTIONS IN PATIENTS OF IDIC-15 SYNDROME, A NEUROLOGICAL RARE DISEASE **CODE** I.16

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ABSTRACT

Introduction: The Idic15 Syndrome is an Autistic Spectrum Disorder (ASD) caused by duplications of the 15q11-q13 region, which contains some genes related to the immune response. An ongoing study with young patients recruited among the members of the Idic15 Spain Association, has shown that about 50% of the patients had repeated upper-respiratory tract infections. In order to provide eventual biomakers of suceptibility to infections in these patients, we have performed polychromatic immunophenotype of relevant lymphocyte subppulations.

Methods: This was a case-control, observational study of 28 patients diagnosed with Idic15, recruited among the members of the Idic15 Spain Association, and 17 controls matched by age, sex and geographical area. The membrane expression of CD3, CD4, CD8, CD14, CD16, CD19, CD21, CD24, CD27, CD28, CD28, CD45RA, CD56, CD57, CD197, CD279, IgD and IgM was determined by flow cytometry (Gallios, Beckman Coulter) using three panels of monoclonal antibodies conjugated with fluorochromes (Duraclone, Beckman Coulter). Cytometric data were compared statistically between the cohorts of controls and patients with- and without frequent infections.

Results: Our results show that the cohort of patients with Idic-15 syndrome have decreased absolute (cells/μL) and relative (percentage of the parental population) concentration in peripheral blood of the subpopulation of central memory CD8 T-cells, of the population of total B cells and isotype-switched memory B lymphocytes. Stratification of the patient cohort according to the clinical history of frequent infections revealed that patients with frequent infections, compared with patients without frequent infections, showed additional decreases in circulating levels of naïve- and central memory CD8 T cells, of total and naïve B lymphocytes, while plasmablast and isotype-unswitched memory B cells increased.

Conclusion: Our study suggests that the increased susceptibility to infections in some Idic-15 patients might be linked to alterations in relevant T- and B-cell subpopulations that could become suitable predictors of infection risk in such patients. Project financed by donations to the "One House One Life" Initiative promoted by Great Chance SLU.

CONFLICT OF INTEREST No potencial conflict of interest to report.

TITLE VALIDATION OF A QUICK FLOW CYTOMETRY-BASED ASSAY FOR ACUTE INFECTION BASED ON CD64 AND CD169 EXPRESSION. **CODE** I.17

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ABSTRACT

Introduction: Several parameters aid in deciphering between viral and bacterial infections; however, new tools should be investigated in order to reduce the time to results and proceed with an early target-therapy. Validation of a biomarker study, including CD64 and CD169 expression, was conducted.

Material and methods: Patients with active SARS-CoV-2 infection (ACov-2), bacterial infection (ABI), healthy controls, and antiretroviral-controlled chronic HIV infection were assessed. Whole blood was stained and, after lysing no-wash protocol, acquired by flow cytometry. The median fluorescence intensity (MFI) of CD64 and CD169 was measured in granulocytes, monocytes, and lymphocytes. The CD64 MFI ratio granulocytes to lymphocytes (CD64N) and CD169 MFI ratio monocytes to lymphocytes (CD169Mo) were evaluated as biomarkers of acute bacterial and viral infection, respectively.

Results: A CD64N ratio higher than 3.3 identified patients with ABI with 83.3 and 85.9% sensitivity and specificity, with an area under the curve (AUC) of 83.5%. In contrast, other analytic or hematological parameters used in the clinic had lower AUC compared with the CD64N ratio. Moreover, a CD169Mo ratio higher than 3.3 was able to identify ACov-2 with 91.7 and 89.8 sensitivity and specificity, with the highest AUC (92.0%).

Conclusion: This work confirms the previous data of CD64N and CD169Mo ratios in an independent cohort, including controlled chronic viral HIV infection patients as biomarkers of acute bacterial and viral infections, respectively. Such an approach would benefit from quick pathogen identification for a direct-therapy with a clear application in different Health Care Units.

CONFLICT OF INTEREST No potencial conflict of interest to report.

TITLE BRAF MUTATION IN MATURE B-CELL NEOPLASMS NON HAIRY CELL LEUKEMIA **CODE** I.18

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ABSTRACT

Introduction: BRAF is a frequently mutated oncogene in a variety of cancers. Presently, BRAF V600E mutation had emerged as a genetic characteristic of hairy cell leukemia (HCL) and is considered to be most useful for differentiating this entity from related lymphomas, as it is exceedingly rare in other Non-Hodgkin Lymphomas (NHL). We present a clinical case in which this mutation was found in a patient with a Diffuse Large B-Cell Lymphoma, illustrating this potential diagnostic drawback.

Methods: A 72-year-old woman, with no relevant previous medical history, turned to a private health care provider with complaints of nausea and vomiting with several days of evolution. Although nocturnal hypersudoresis was referred, no other B symptoms were reported. At clinical evaluation, she was hemodynamically stable, with no fever, and a hepatosplenomegaly was observed. Analytically, a slight anemia with trombocytopenia and elevated LDH. The abdominal CT scan revealed a liver of increased dimensions and a marked splenomegaly with suspected splenic rupture, with infracentimetric retroperitoneal ganglia. The patient was then transferred and admitted to an experienced tertiary cancer centre to perform a splenectomy and to proceed with clinical investigation.

Results: Initial flow cytometric immunophenotypic characterization of peripheral blood presented with 32.94% of pathological B lymphocytes, but the immunophenotype and proliferative index (intermediate) were not suggestive of HCL. Then, the immunophenotypic characterization of tumor fragment (spleen) revealed the presence of a B-NHL with a overlapping immunophenotype from previous B-NHL described in peripheral, but a higher proliferative index, suggesting an high grade B-cell lymphoma. The morphological and immunohistochemical analysis of the anatomical piece revealed findings compatible with the diagnosis of a Diffuse Large B-cell Lymphoma, although the possibility of transformation / progression of a previous indolent B lymphoma was admitted. Research on mutations in the BRAF gene was performed using real time PCR, and the oncogenic variant V600E/D of the BRAF gene was detected.

Conclusion: The immunophenotypical analysis of this patient presented with a peculiar heterogeneity, with peripheral blood pathological B cells not presenting any characteristic pattern of any B-NHL but a clear diagnosis of a Diffuse Large B-Cell Lymphoma in the spleen fragment. Within diverse mature B-cell neoplasms, the BRAF V600E mutation is highly associated with hairy cell leukemia and can be considered a driver mutation of this disease. In contrast, within literature, there are not many reported cases of other types of NHL-B with this specific mutation present. The aim of this report is to raise awareness of the possibility of the presence of this mutation in other B-cell neoplasms, not only because of the potential misdiagnosis, but also as targeted inhibition of activated BRAF is now possible.

CONFLICT OF INTEREST No potencial conflict of interest to report.

TITLE COLONIC AND CIRCULATING T-CELL SUBSETS IN INFLAMMATORY BOWEL DISEASE **CODE** I.19

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ABSTRACT

Introduction: Inflammatory bowel disease (IBD) is a group of immune-mediated diseases with uncertain pathobiology. Considering the scarcity of data on the local immune compartment in IBD patients, we aimed to characterize and evaluate possible differences in T-cell subsets from peripheral blood (PB) and colon biopsies of patients with primary sclerosing cholangitis (PSC), Crohn's disease (CD) and ulcerative colitis (UC), and compare them with non-IBD patients.

Methods: We enrolled a cohort of patients with PSC (n=6), CD (n=16), and UC (n=16), diagnosed according to international criteria, and a healthy control group (HC), non-IBD, assessed for colorectal cancer screening, constipation, or irritable bowel syndrome (n=12). IBD patients with endoscopic disease activity were excluded. Colonoscopy was performed in all patients and colonic biopsies collected from non-inflamed areas. PB samples were collected at the same time. All samples were processed up to 5h after collection. T-cell subsets from colonic and PB samples of each participant were evaluated by multiparametric flow cytometry (MFC) using the following mAbs: CD3, CD4, CD25, CD39, CD45, CD45RA, CD127, and CD197. All samples were acquired in an 8-colour BD FACS Canto IITM and analysed with Infinicyt 2.0TM.

Results: Groups showed no differences for age and gender. Assessing PB and colon as paired samples, we found positive correlations between local and circulating subsets of naïve and memory CD4+T cells, naïve, memory and CD39+ memory Tregs ($r \geq 0.349$; $p \leq 0.019$), but not for the mother populations (i.e. total Treg, CD4+ and CD4- T-cells). Despite groups had no significant differences for cell subsets, we identified a normal profile in HC, with lower percentages of colonic CD4+T-cells compared to PB. In addition, both colonic CD4+ and CD4- T-cell subsets were mostly composed by memory cells (90.3% and 95.2%, respectively), with few naïve cells, and very low to absent effector cells. Colon samples were also richer in Tregs than PB, and particularly in CD39+memory Tregs. UC patients presented a similar profile, but they also showed increased CD39+naïve Tregs in colon compared to PB. Interestingly, CD patients presented a distinctive feature with similar percentages of colonic and PB CD4+ and CD4- T-cells, thus with higher colon CD4/CD8 ratios than HC and UC. Finally, the PSC group presented a profile closer to DC patients, with similar colonic and PB CD4+ and CD4- T-cell percentages, showing also increased memory subsets and Tregs in colon.

Conclusion: Our study, using for the first-time MFC in the characterization of colonic T-cell subsets in PSC patients, made it possible to establish significant differences between local and circulating populations, namely Tregs, their expression of CD39 and the differentiation patterns of naïve, memory and effector T-cells. Besides bringing new insights into the local immune subsets in IBD, this approach may have future applications in patient monitoring.

CONFLICT OF INTEREST No potencial conflict of interest to report.

TITLE A VALIDATION STUDY OF A FLOW CYTOMETRIC ASSAY FOR RAPID DETECTION OF ESBL IN ENTEROBACTERALES GROUP II **CODE** M.01

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ABSTRACT **Introduction:** The ability of bacteria to produce ESBL (extended spectrum beta-lactamases enzymes) is well known, as well as its clinical implications. Their detection in clinical laboratories is normally performed in Enterobacterales group unlike Enterobacterales group II such as Serratia spp, Enterobacter spp, Citrobacter freundii, Morganella morganii and Providencia stuartii. EUCAST proposed a protocol for its detection and based on it, a flow cytometric assay was developed by FASTinov, a spin-off of Porto University, Portugal. A two-site validation of a rapid assay was performed, namely at FASTinov lab, in Porto University and at Ramon et Cajal Hospital Microbiology lab in Madrid.

Material and methods: One-hundred seventeen clinical isolates belonging to Enterobacterales group II were studied (43 at FASTinov and 74 at Ramon et Cajal Hospital). All the isolates included were categorized as resistant R) or susceptible increased exposure (I) to cefotaxime or ceftazidime. To test for the presence of an ESBL, susceptibility to cefepime with and without clavulanic acid by disk diffusion tests (Oxoid, Thermofisher) was performed and considered the reference method. In parallel, bacterial cells in exponential growth-phase were incubated for 1 h, 37ºC with cefepime at several concentrations, with and without clavulanic acid, and stained with a membrane-potential sensitive fluorescent probe. The intensity of fluorescence of the bacterial cells was quantified in a flow cytometer (CytoFlex, Beckman Coulter) and analyzed through a proprietary software. Whenever an increase of the fluorescence of the cells incubated with cefepime and clavulanic acid in comparison with cells without clavulanic acid was registered, the strain was considered positive for ESBL. The proportion of agreement (PA), sensitivity and specificity of the assay were calculated.

Results: Thirty-four clinical strains were classified as ESBL positive by reference method. PA was 90.6% with sensitivity and specificity of 94.1% and 89.2% respectively.

Conclusion: The hereby described flow cytometric assay is very promising in Microbiology labs allowing a rapid detection of ESBL in a simple and fast approach. A rapid detection of such enzymes will improve therapeutic management but also allow the isolation of the patient in useful time, preventing the spread of those resistant bacteria through hospital/community.

CONFLICT OF INTEREST No potencial conflict of interest to report.

TITLE A NEW METHOD FOR RAPID DETECTION OF AMPC BASED ON FLOW CYTOMETRY **CODE** M.02

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ABSTRACT **Introduction:** AmpC plasmidic are common and widely distributed but not usually detected on the routine clinical microbiology laboratory. EUCAST protocols established its phenotypic detection but it is a growth dependent method, takeing long time to give results. FASTinov, a spin-off of Porto University, in Portugal, developed a new and disruptive approach for rapid antimicrobial susceptibility assay, based on flow cytometric analysis. Additionally, a new assay for AmpC detection was developed. A two-site study aiming to validate the detection of AmpC enzymes on Enterobacterales group I using flow cytometry technology is hereby described.

Material and methods: A total of 95 clinical strains belonging to Enterobacterales group I (E. coli, Kl.pneumoniae and Proteus mirabilis) that screened positive for AmpC i.e., resistant to cefotaxime and/or ceftazidime and simultaneously resistant to cefoxitin, were selected on 2 sites: FASTinov lab (n=61) and at the Department of Microbiology of Hospital Ramon et Cajal in Madrid, Spain (n=34). On both labs the strains were tested in parallel by disk diffusion phenotypic assays (ROSCO Diagnostic, Denmark) and by the new method developed by FASTinov. An exponential growth phase inoculum of each strain was incubated with cefotaxime, with and without cloxacillin and ceftazidime, with and without cloxacillin, during 1 h at 37ºC. Together with the antibiotic drugs, a membrane-potential sensitive fluorescent probe was included and then analyzed in a flow cytometry (CytoFlex from Beckman Coulter). The increase of the intensity of the fluorescence of the cells with cloxacillin compared with cells without cloxacillin was considered a synergic effect i.e. positive for the presence of AmpC. The flow cytometric results were compared with results obtained with the disk diffusion, considered the reference method.

Results: Forty-six clinical strains were classified as AmpC positive by reference method; the proportion of agreement with the flow cytometric assay was 92.6%. The sensitivity and the specificity was 95.6% and 90% respectively.

Conclusion: A rapid method for AmpC plasmidic detection is hereby described with great accuracy that could impact both on patient´s treatment as well as on public health control, since this kind of enzymes are easily spread.

CONFLICT OF INTEREST No potencial conflict of interest to report.

TITLE INCONSISTENCY OF FLOW CYTOMETRIC VIABILITY TESTS BASED ON MEMBRANE PERMEABILITY AND MEMBRANE DEPOLARISATION WHEN EXAMINING OXIDATIVE TOXICITY IN E. COLI STRAINS DEFICIENT IN ANTIOXIDANT GENES **CODE** M.03

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ABSTRACT **Introduction:** A common procedure in flow cytometry (FCM) of microorganisms is the analysis of the cytotoxic effects of chemical compounds. Most bacterial viability studies assess membrane permeability to nucleic-acid binding dyes, SYTO-9 and propidium iodide (PI) being the most widely used. Both live and dead bacteria are permeable to SYTO-9 emitting green fluorescence. PI permeates dead or damaged cells, emitting orange fluorescence. Viability of bacteria may be also assessed by dual staining with PI and membrane-potential sensitive probes, such as bis-(1,3-dibutylbarbituric) trimethine oxonol [DiBAC4(3)] that permeates through depolarised membranes. Such assays allow detecting changes in membrane permeability to small ions which may be lethal in the absence of evident membrane lesions. E. coli B WP2 strains with spontaneous deficiencies in wall lipopolysaccharides are permeable to small- and mid-size organic chemicals and fluorochromes. We have inactivated several of their key genes involved in antioxidant defence, generating suitable biosensors of oxidative damage (Herrera et al., Curr. Protocols Cytometry 24 (1), 2003).

Methods: WP2 strains IC188 (control) and IC5232 (superoxide dismutase deficient) were incubated with different prooxidants (Sigma) generating either superoxide anion (paraquat, menadione) or peroxides (tert-Butyl Hydroperoxide). Bacterial viability was assesed on a Gallios flow cytometer (Beckman-Coulter). using BacLight LIVE/DEAD kit (Thermo Fisher Scientific), based on SYTO-9 and PI, or by dual staining with DiBAC4(3) and PI (Thermo Fisher). Bacterial growth was assessed by measuring optical density of bacterial preparations at 600nm absorption (OD600).

Results: Bacterial growth OD600 curves showed that all prooxidants tested inhibited bacterial growth even at low doses, more evident on the antioxidant-deficient strain. Compounds generating superoxide caused a decrease in viable cells reflected by decresaed number of live cells with polarised membrane (DiBACneg PIneg) and increase of depolarised cells with apparently non-permeabilized membrane (DiBACpos PIneg). In these conditions, live cells (SYTO9pos PIneg) diminished and an intermediate population (SYTO9neg PIneg) was increased. In superoxide-induced toxic conditions, canonical PIpos dead cells (i.e., DiBACposPIpos or SYTO9negPIpos) were not detectable. In contrast, in peroxide-induced cytotoxicity compounds, cell growth inhibition was associated with appeareance of canonical dead cells in both assays of viability.

Conclusions: Although the biological basis of the described discrepancy needs to be elucidated, our results show that, at least in bacteria, the specific mechanisms of oxidative cytotoxicity of different prooxidants may lead to ambiguous results with simple assays of viability. This supports that studies of cytotoxicity in bacteria should combine analysis of both membrane depolarization and permeabilization to define better cell viability and death.

CONFLICT OF INTEREST No potencial conflict of interest to report.

TITLE EVALUATION OF CELL DEATH MECHANISM AND CELL CYCLE PROGRESSION OF SH-SY5Y CELLS EXPOSED TO ZEARALENONE´S METABOLITES AND BEAUVERICIN **CODE** O.01

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ABSTRACT There are a wide variety of stimuli and conditions, both physiological and pathological, that can trigger cell cycle progression and programmed cell death, but not all cells will necessarily die in response to the same stimulus. One of these triggers which have been wildly studied are mycotoxins. These compounds are produced by Fusarium species and have demonstrated cytotoxicity and neurotoxicity through impairing cell proliferation, gene expression and induction of oxidative stress. The aim of present study was to analyze the cell cycle progression and cell death pathway by flow cytometry in undifferentiated SH-SY5Y neuronal cells exposed to α-zearalenol (α-ZEL), β-zearalenol (β-ZEL) and beauvericin (BEA) over 24h and 48h individually and combined at the following concentration ranges: from 1.56 to 12.5 μM for α-ZEL and β-ZEL, from 0.39 to 2.5 μM, for BEA, from 1.87 to 25 μM for binary combinations and from 3.43 to 27.5 μM for tertiary combination. Alterations in cell cycle were observed remarkably for β-ZEL at its highest concentration in all treatments where engaged (β-ZEL, β-ZEL + BEA and β-ZEL + α-ZEL), for both 24h and 48h by activating the cell proliferation in G0/G1 phase (up to 43.6%) and causing delays or arrests in S and G2/M phases (up to 19.6%). Tertiary mixtures revealed increases of cell proliferation in subG0 phase by 4-folds versus control. Similarly, for cell death among individual treatments β-ZEL showed a significant growth in early apoptotic cells population at highest concentration assayed as well as for all combination treatments where β-ZEL was involved, in both early apoptotic and apoptotic/necrotic cell death pathways.

Keywords: cell cycle, cell death, Fusarium mycotoxins, flow cytometry

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CONFLICT OF INTEREST No potencial conflict of interest to report.

TITLE BRONCHOALVEOLAR LAVAGE ABNORMAL DNA CONTENT: WHAT CAN WE EXPECT? **CODE** O.02

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ABSTRACT **Introduction:** Bronchoalveolar lavage (BAL) is a useful method of respiratory tract investigation. The lavage of part of the lung allows harvesting cellular and non-cellular compounds of this space. Although inflammatory cells like macrophages, lymphocytes, neutrophils and eosinophils are more prevalent, additional different abnormal cells can be found in case of pulmonary malignant pathology. Currently, flow cytometry analysis has been widely used in the identification of the subpopulations of lymphoid cells. Additionally, DNA analysis can be an aid in the identification of hematopoietic neoplasms as well as primary lung cancer and other metastatic lung tumours. In this study we evaluate the viability of BAL flow cytometric analysis in finding malignant cells.

Methods: Between 2010 and 2020, 193 samples of BAL from oncology patients were analysed by flow cytometry for the identification of lymphoid cells subpopulations. In addition, samples with clinical history of solid tumour or in those cases where a CD45- population was detected (N=42) were further characterised using a pan-cytokeratin antibody. Whenever cytokeratin+ population was found, DNA content evaluation was performed using the Ber-EP4 antibody together with DRAQ5 dye. The results obtained by flow cytometric analysis were then compared with the cytology.

Results: From the 42 studied cases, DNA content was not possible in 16 due to the low number of Ber-EP+, CD3-, CD14-, CD45- cells present. The remaining 26 cases showed an abnormal DNA content, with a median DNA Index (DI) 1.55 (min. 1.33 – max 2.48) and 77% of the cases had a DI between 1.4 e 1.6. S phase fraction had a median of 6.40 (min 0.01% – max 51%). However, in only 5 cases cytologic studies confirmed the presence of tumour cells (3 lung adenocarcinoma, 1 gastric adenocarcinoma and 1 larynx spinocellular carcinoma).

Conclusion: Cells with abnormal DNA content are usually associated with malignant processes which makes flow cytometric analysis a useful aid in finding pathological cells. In our study, DNA ploidy was hard to validate due to the lack of specific cell markers that would make the match of the aneuploid peak to the tumour cells since BAL cells, such as macrophages and other high proliferative cells, may have an aneuploid/hyperdiploid DNA content. This phenomenon can explain why in only 5 cases malignant cells were confirmed by cytology. We believe that DNA content evaluation by flow cytometric analysis combined with more tumour specific antibodies such as an anti-cytokeratin specific or the thyroid transcription factor-1, could play a major role in the characterization of the tumour microenvironment in BAL specimens.

CONFLICT OF INTEREST No potencial conflict of interest to report.

TITLE NUCLEOLIN-SLEA AS E-SELECTIN LIGANDS AND POTENTIALLY TARGETABLE BIOMARKERS AT THE CELL SURFACE OF GASTRIC CANCER CELLS: A FLOW CYTOMETRY-ASSISTED APPROACH **CODE** S.01

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ABSTRACT Gastric cancer (GC) is a major health burden worldwide, with half of patients developing metastases within 5 years after treatment, urging novel biomarkers for diagnosis and efficient therapeutic targeting. Sialyl-Lewis A (SLeA), a terminal glycoepitope of glycoproteins and glycolipids, offers tremendous potential towards this objective. It is rarely expressed in healthy tissues and blood cells, while it is present in highly metastatic cell lines and metastases. SLeA is also involved in E-selectin mediated metastasis, making it an ideal target to control disease dissemination.

Methods and Results: To improve cancer specificity, we have explored the SLeA-glycoproteome of six GC cell models, with emphasis on glycoproteins showing affinity for E-selectin through a flow cytometry-assisted approach. A novel bioinformatics-assisted algorithm identified nucleolin (NCL), a nuclear protein, as a potential targetable biomarker potentially involved in metastasis. The flow cytometry reinforces the probability of NCL expression on the cell surface. Moreover, several immunoassays, including Western blot and in situ proximity ligation fortified the existence of cell surface NCL-SLeA glycoforms in GC. The NCL-SLeA glycophenotype was associated with decreased survival and was not reflected in relevant healthy tissues. Conclusions: NCL-SLeA is a biomarker of poor prognosis in GC holding potential for precise cancer targeting. The flow cytometry analysis highlighted the importance of currently available clinical tools in biomarker discovery. In summary, this is the first report describing SLeA in preferentially nuclear protein, setting a new paradigm for cancer biomarkers discovery and targeted therapies.

CONFLICT OF INTEREST No potencial conflict of interest to report.

TITLE CD39 AND CD73 EXPRESSION ON T CELLS AND TUMOR CELLS IN BLADDER CANCER **CODE** S.02

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ABSTRACT **Introduction:** Emerging evidence suggests that tumors generate adenosine in tumor microenvironment (TME), inhibiting effector function of multiple immune cell subsets, thereby allowing neoplastic growth. This is dependent on the adenosinergic pathway (AP), in which CD73 and, more recently CD39, seem to play a key role. We aim to quantify and characterize the phenotype of different subpopulations of T cells (CD4+, CD8+ and Treg) at tumor microenvironment, in surrounding non-malignant tissue and in peripheral blood and, in parallel, evaluate the expression of CD39 and CD73 in urothelial bladder cancer (BC) cells. This is part of a larger study aiming to trace an immunologically-based signature of the AP in BC, with therapeutic and prognostic purposes.

Methods: We conducted a study with 24 patients with histological confirmed urothelial carcinoma of the bladder, with indication for surgery – transurethral resection of the bladder or radical cystectomy. Peripheral blood, tumor and normal-appearing matching tissue were sampled and analyzed by flow cytometry, with a FACSCanto (R) II cytometer. A systemic functional evaluation of the immune and adenosinergic systems, with regard to the subpopulations of T cells and adenosinergic pathway (CD39; CD73) was performed.

Results: Compared to the normal matching bladder tissue, the immunophenotype of BC tissue was characterized by a specific profile of T cell infiltration: increased CD4+ (44.7 vs. 32.3%) and decreased CD8+ (52.3 vs. 66.1%) T cells. Most notably, BC exhibited a marked increase of regulatory T cells (CD4+, CD25+bright, CD127+dim)(18.6 vs. 6.4%, p= 0.008). The majority of T cells, particularly in tumor and normal tissues, had the CD39+/CD73- phenotype. We found an evident increase on the expression of CD39 in all subpopulations of T cells, (CD4+, CD8+ and Treg) either with an activated phenotype (HLA-DR+ and/or CD25+) or not, reaching a mean factor of 20.0 x, when comparing tumor microenvironment to peripheral blood, and 8.6 x compared to normal matching tissue. There was a significant correlation between the percentage of CD4+ Treg cells and the expression of CD39, not only in peripheral blood (p=0.005), but also in normal tissue (p=0.005) and tumor tissue (p=0.018). The same correlation occurred for CD8+ T cells, but only in tumor tissue (p=0.012). In turn, CD73 expression is mostly associated with tumor cells, as 53.6 ± 13.2% of tumor cells express this enzyme.

Conclusion: Our results point to an immunosuppressive tumor microenvironment in bladder cancer, with a decreased infiltration of cytotoxic T cells and an increase of Treg subpopulations, which seems to be associated with an amplified activity of the adenosinergic pathway, where T cells (expressing CD39) and tumor cells (expressing CD73) apparently play a complementary role.

CONFLICT OF INTEREST No potencial conflict of interest to report.

TITLE PERIPHERAL LYMPHOCYTE SUBPOPULATIONS IN PROSTATE CANCER - DATA FROM AN ANIMAL MODEL **CODE** S.03

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ABSTRACT **Introduction:** Prostate cancer (PCa) is one of the most common cancers among men worldwide. The presence of immune cells in human cancer raises a fundamental question in oncology. The interaction between immune system and PCa is an important field for translational research. This work aimed to characterize the peripheral lymphocyte subpopulations in a PCa animal model.

Methods: Twenty-five male Wistar Unilever rats (Rattus norvegicus) with twelve weeks of age were randomly divided into two groups: Control (n=10) and Induced (n=15). All procedures were approved by the Portuguese Competent Authority (DGAV no. 021326). Prostate lesions were induced through the administration of flutamide (50 mg/kg, TCI Chemicals, USA), testosterone propionate (100 mg/kg, TCI Chemicals, USA) and N-methyl-N-nitrosourea (30 mg/kg, Sigma Chemical Co., Spain), and crystalline testosterone implants. Animals were humanely sacrificed at 61 weeks of age. Peripheral blood of all animals was collected by intracardiac puncture and transferred into tubes containing EDTA salt as an anticoagulant for flow cytometry analysis. The following conjugated monoclonal antibodies were used: cyCD3-BV421, CD3-FITC, CD25-APC, CD45-BV510, CD127-PE, CD161-FITC, CD4-PE/Cy7, CD45RA-APC/Cy7, OX-82-PE and CD8a-PerCP. The flow cytometry immunophenotyping was performed in a BD FACSCantoTM II cytometer (BD Biosciences, USA) and data were analysed with InfinicytTM, flow cytometry software 1.7 version. Statistical analysis was performed using SPSS 25. The differences were considered statistically significant at p0.05). Similarly, CD8+ lymphocyte population was higher in control group than in induced group (9.56±0.74 vs 6.38±0.32) (p<0.05). Inversely, the population of regulatory T cells (TRegs) (2.99±0.46 vs 4.630±0.35), the TRegs/CD8 ratio (0.35±0.09 vs 0.45±0.08) and the TRegs/Natural Killer ratio (0.52±0.05 vs 1.03±0.13) were higher in induced group when compared with control one (p<0.05).

Conclusion: The population of Tregs increased in induced animals, while the population of NK decreased in these animals, which is in accordance with data previously published by other authors reporting the increase of Tregs and decrease of NK cells in animals with cancer. The characterization of these immune system subpopulation can be important for other studies such as preclinical cancer models.

CONFLICT OF INTEREST No potencial conflict of interest to report.

TITLE EFFECT OF EXERCISE TRAINING ON LYMPHOCYTE SUBPOPULATIONS IN CHEMICALLY AND HORMONALLY INDUCED PROSTATE CANCER: FLOW CYTOMETRY ANALYSIS **CODE** S.04

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ABSTRACT **Introduction:** Long-term and regular exercise training is suggested to have an immunomodulatory effect, protecting against several diseases. This work aimed to analyse the effect of exercise training on peripheral lymphocyte subpopulations in a model of prostate cancer (PCa) chemically and hormonally induced.

Methods: Fifty-five male Wistar Unilever rats of 4 weeks of age were randomly divided into four experimental groups as follow: control sedentary group (SED+CONT; n=10), control exercised group (EX+CONT; n=10), induced sedentary group (SED+PCa; n=15) and induced exercised group (EX+PCa; n=20). Prostate lesions were induced through the sequential administration of flutamide (50 mg/kg, TCI Chemicals, USA), testosterone propionate (100 mg/kg, TCI Chemicals, Portland, USA) and N-methyl-N-nitrosourea (30 mg/kg, Sigma Chemical, Spain), and subcutaneous implantation of tubes filled with crystalline testosterone (Sigma Chemical, Spain). At eight weeks of age, exercised animals started the training in a treadmill (Treadmill Control LE 8710, USA), 5 days/weeks, for 53 weeks. Animals were sacrificed at 61 weeks of age through an intraperitoneal injection of ketamine (75 mg/kg, Imalgene® 1000, Merial S.A.S., France) and xylazine (10 mg/kg, Rompun® 2%, Bayer Healthcare S.A., Germany), followed by exsanguination by cardiac puncture. Peripheral blood of all animals was collected by intracardiac puncture and transferred into tubes containing EDTA salt as an anticoagulant for flow cytometry analysis. The following conjugated monoclonal antibodies were used: cyCD3-BV421, CD3-FITC, CD25-APC, CD45-BV510, CD127-PE, CD161-FITC, CD4-PE/Cy7, CD45RA-APC/Cy7, OX-82-PE and CD8a-PerCP. The flow cytometry immunophenotyping was performed in a BD FACSCantoTM II cytometer (BD Biosciences, USA) and data were analysed with InfinicytTM, flow cytometry software 1.7 version. The prostate was collected and stained with H&E for histopathological analysis. Statistical analysis was performed using SPSS 25. The differences were considered statistically significant at $p<0.05$.

Results: A higher level of CD161+NK cells were observed in EX+PCa group when compared with SED+PCa group ($p<0.05$). These results are in accordance with the literature which suggests that exercise training increase NK cells number. Moreover, long-term exercise training increased gama delta Tcells/CD3 ratio and decreased Treg/NK ratio in PCa-induced groups (p0.05).

Conclusion: These results reinforce the beneficial role of exercise in anti-tumour immune response. Additional studies are warranted to better understand these results.

CONFLICT OF INTEREST No potencial conflict of interest to report.

TITLE UTILITY OF CD326 TO DETECT EPITHELIAL CELLS IN SEROUS FLUIDS **CODE** S.05

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ABSTRACT The suspension cells in the serous fluids are essentially leukocytes and mesothelial cells. In cases of malignancy, tumor cells infiltrate the serous space through direct or lymphatic dissemination. Cytopathology, aided by immunocytochemistry, is considered the “gold standard” in the detection of malignant cells in effusions. CD326 (epithelial-specific antigen, clone Ber-Ep4) is a cell membrane glycoprotein on human epithelia. It is expressed by a large range of epithelial tumors, including skin, gastrointestinal, breast, and tumors of male and female genitourinary tract. A presence of epithelial cells in the body fluid should raise the suspicion of metastatic epithelial malignancy.

With this work, we assessed the interest in the investigation of CD326 positive cells in serous fluids through flow cytometry in a routine way in our hospital. The clinical pathology and the surgery services of our institution studied 32 serous fluids with clinical suspicion of a neoplastic etiology. 26 ascitic fluids and 6 pleural fluids, 13 female and 19 males with age comprised between 47 and 87 years and mean age of 69 years. All samples were evaluated, by flow cytometry and cytopathology. To 500 µL of serous fluid sample was added the monoclonal antibodies: 5µL of CD326 FITC, 10µL of CD33 PE, and 5µL of CD45 APC. After an incubation period of 15 min in the dark was added 2 ml of FacsFlow, centrifuged, and resuspended in 500 µL of FacsFlow. The acquisition was performed in a FACSCalibur; in a first step acquired all the events, in a second step performed a gate in CD45 negative events and acquired until 500 thousand events. The results were analyzed by the InfinicytTM 1.8. We search the presence of CD45-CD33-CD326+ cells; that identification leaves us to presume that positive sample for malignant epithelial cells and their absent negative. The results of the flow cytometry were compared with the cytopathology reports demonstrated a sensitivity of 92% and specificity of 89%. Of the 32 samples analyzed, 30 samples (94%) had concordant results and 2 samples (6%) discordant results. The discordant samples were ascitic fluid, one false negative and one false positive.

In the future is needed to establish the minimum value of frequency of CD45-CD33-CD326+ cells to assume a positive sample for malignant epithelial cells. Some samples have a low number of cells, to overcome this limitation must acquire the largest possible number of cells. The detection of CD45-CD33-CD326+ cells by flow cytometry is strongly indicative of the presence of malignant epithelial cells, presenting, in our study, a sensitivity of 92% and specificity of 89%.

Our work clearly shows that study of the CD326 expression by flow cytometry in effusions can be a useful method to identify non-hematological cells of epithelial origin in a routine laboratory.

CONFLICT OF INTEREST No potencial conflict of interest to report.

TITLE THE CYTOTOXIC POTENTIAL OF STARCH-CAPPED SILVER-NANOPARTICLES (AGNPS) AND THEIR ABILITY TO INDUCE CELL CYCLE ARREST IN PROSTATE CANCER CELLS **CODE** S.06

AUTHORS NAMES Morais M.[1,2,3*]; Machado V.[1*]; Dias F.[1]; Palmeira C.[4,5,6]; Martins G.[4,5]; Fonseca M.[7]; Martins C.[7]; Prior J.[7]; Teixeira A. L.[1]; Medeiros R.[1,2,3,6,8]

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ABSTRACT **Introduction:** Prostate Cancer (PC) is the second most frequent cancer diagnosed in men and the fifth leading cause of death worldwide. The androgen deprivation therapy (ADT) is a therapeutic approach frequently used in advanced stages of the disease. However, patients usually develop resistance within 2-3 years, progressing to Castration Resistant PC (CRPC). In the last years, new drugs with increased sensitivity or aiming to new targets came up but also showed limited benefits leading to patients' relapse. Thus, overcoming treatment resistance remains a major challenge in PC patients' management, making the development of new therapeutic approaches of great importance. Silver nanoparticles (AgNPs) synthesized through green approaches have been studied as anticancer agents because of their specific physical-chemical properties. Their effects are highly dependent on shape, size, and composition, as well as on cells' characteristics, suggesting a specific-cell type effect. According to literature, AgNPs can exert their effect in cell membrane, nucleus and mitochondria leading to cell cycle arrest and ultimately to apoptosis. This study explored the cytotoxic capacity of starch-capped AgNPs, synthesized through green methods, in LNCaP and PC-3, a hormonal-sensitive and hormone-resistant PC cell line, respectively.

Methods: AgNPs were synthesized in a microwave pressurized synthesizer and characterized by Ultraviolet-Visible (UV-Vis) spectroscopy, Transmission Electron Microscopy (TEM) and Energy-dispersive X-ray spectroscopy (EDX). Their cytotoxicity was assessed regarding their ability to alter morphological aspect (optical microscopy), induce damage in cytoplasmic membrane (Trypan Blue Assay), mitochondria (WST-1 assay), cellular proliferation (BrdU assay) and cell cycle (Propidium iodide and flow-cytometry).

Results: AgNPs showed Surface Plasmon Resonance (SPR) of approximately 408 nm and average size of 3 nm. They successfully altered the cells' morphology, inducing damage in cytoplasmic membrane and mitochondria, at concentrations equal and above 20 ppm. After treatment with AgNPs, there was a significant increase of the number of cells in G0/G1 phase and decrease of cells in the G2/M phase for the LNCaP cell line. In the PC-3 cell line, there was a significant decrease of the number of cells in G0/G1 phase and increase of cells in the G2/M phase.

Conclusion: Starch-AgNPs showed potential as anticancer agents in PC. They were able to induce cytoplasmic and mitochondrial damage, leading to cell cycle arrest in G0/G1 and G2/M, blockage of proliferation and consequent death in LNCaP and PC-3 cells, respectively. The arrest in different phases of the cell cycle may be due to PC-3's more aggressive phenotype which allows them to progress more in the cell cycle. However, their arrest in G2/M phase may be associated with the G2-M DNA damage checkpoint, which ensures that cells do not initiate mitosis until damages are sufficiently repaired.

CONFLICT OF INTEREST No potencial conflict of interest to report.

ABSTRACTS

PUBLICATIONS

TITLE MONOCYTES/MACROPHAGES PROFILE DETERMINATED BY FLOW CYTOMETRY AS A BIOMARKER TO PATHOLOGICAL INFLAMMATION IN PATIENTS WITH COMMON VARIABLE IMMUNODEFICIENCY

AUTHORS NAMES Kauzar Mohamed Mohamed[1]; Kissy Guevara-Hoyer[1]; Marcos Viñuela[1]; Eduardo de la Fuente[1]; Natalia Rodríguez Vicente[1]; Bárbara Alonso Arenilla[1]; Rebeca Pérez de Diego[2]; Miguel Fernández-Arquero[1]; Silvia Sánchez-Ramón[1]

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ABSTRACT **Background:** Common variable immunodeficiency (CVID), the most common symptomatic primary immunodeficiency, comprising a heterogeneous group of patients with hypogammaglobulinaemia. Monocyte dysfunction may be important for immunopathogenesis in subgroups of patients with primary hypogammaglobulinaemia

Methods: We compared the immunological profile of monocytes-macrophages in 4 CVID patients with different clinical phenotypes (hematologic malignancy, cytopenias, unexplained persistent enteropathy, no other disease-related complications (previously “infections only”) and a healthy control to the Hospital Clínico San Carlos, Madrid, Spain.

Results: In the immunological study, the patient with hematological malignancy showed a marked increase in the percentage of intermediate monocytes (32.45%). The patient with unexplained persistent enteropathy, showed intermediate levels of activation in this monocyte subpopulation (12.4%), as did the patient with cytopenia (10.5%). the CVID patient who associated the "infections only" phenotype presented normal expression levels (4.5 %), similarly to the healthy control 4.6%.

Conclusion: The analysis of monocytes-macrophages profile in CVID patients through flow cytometry could identify early mediators hyper-inflammatory response associated with a severe presentation of the disease behaves as a prognostic biomarker.

CONFLICT OF INTEREST No potencial conflict of interest to report.

TITLE A CASE OF CD157 NEGATIVE NON-PAROXYSMAL NOCTURNAL HEMOGLOBINURIA WITH THE CLONE SY11B5

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ABSTRACT **Introduction:** CD157 (BST1 gene)is a glycosylphosphatidylinositol(GPI)-linked molecule expressed in the cell surface of monocytes and neutrophils. It is a useful flow cytometric marker for the analysis and diagnosis of paroxysmal nocturnal hemoglobinuria (PNH). It has been described that monoclonal anti-CD157 antibody clone SY11B5,used in the routine clinical practice, fails to detect thesingle nucleotide polymorphism (SNP) of BST1(p.Arg145Gln).RF3, another commercially available clone, has shown to be able to recognize this SNP. Nevertheless, some authors do not consider this explanation valid in all cases, andhaveproposed otherpossibilities, such as the interference of certain drugs with monoclonal anti-CD157 antibodies. We present a case of CD157-negative non PHN patient in which we will try to elucidate the reason for this particularphenomenon.

Methods: A blood sample (BS) of an87 years old woman was studied by consensus PHN monoclonal panel in a FACSCanto II flow cytometer, Becton Dickinson (BD): FLAER-FITC (Cedarlane), CD157-PE SY11B5 clone (BD), CD10-PECy7 (Beckman-Coulter), CD64-APC (BD), CD14-APCH7 (BD), CD24-Vioblue (MiltenyiBiotec), CD45-HV500 (BD). After a few weeks, a new BSwas analyzed with the initial panel and another with the CD157-FITC RF3 clone (Beckman-Coulter) We sequenced BST1gene of our patient looking for the recently described SNP (p.Arg145Gln) using the following primers: Forward 5’-AGAACGTTCAGTETEGTTGTGCAG-3’, Reverse 5’-GCAGAGTTGTGCAAAGTTGAGA-3’. Sanger sequencing was done with Applied BioSystems® 3130 Genetic Analyzer.

Results: At the time of the first study, normal expression of FLAER, CD24 and CD14, but not binding of anti-CD157 SY11B5 clone was observed on monocytes and granulocytes. The patient hadrecently undergonetreatmentwith intravenous immunoglobulins. Thus, in order to avoid possible interferences, the lack of detection of CD157 with clone SY11B5 was confirmed weeks later in a new BS. However, the RF3 clone detected CD157 expression. It was considered that the presence of the recently described SNP (p.Arg145Gln) could explain these results.Sanger sequencing was done to demonstrate the existence of this SNP in the BST1 gene.

Conclusion: In the presented case of CD157-negative non PHN, no interferences were detected between recent intravenous immunoglobulin treatment and the results of the flow cytometric analysis. The lack of detection of CD157 only seemed to affect testing with SY11B5 clone,having no impact ontesting with RF3 clone. It has been hypothesized that the most probable explanation for this particular phenomenon is the presence of the SNP (p.Arg145Gln). Note to reviewers Sanger sequencing is being done at the moment of the abstract submission’s deadline. Results will be available before XVII Congress of the Iberian Society of Cytometry.

CONFLICT OF INTEREST No potencial conflict of interest to report.

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