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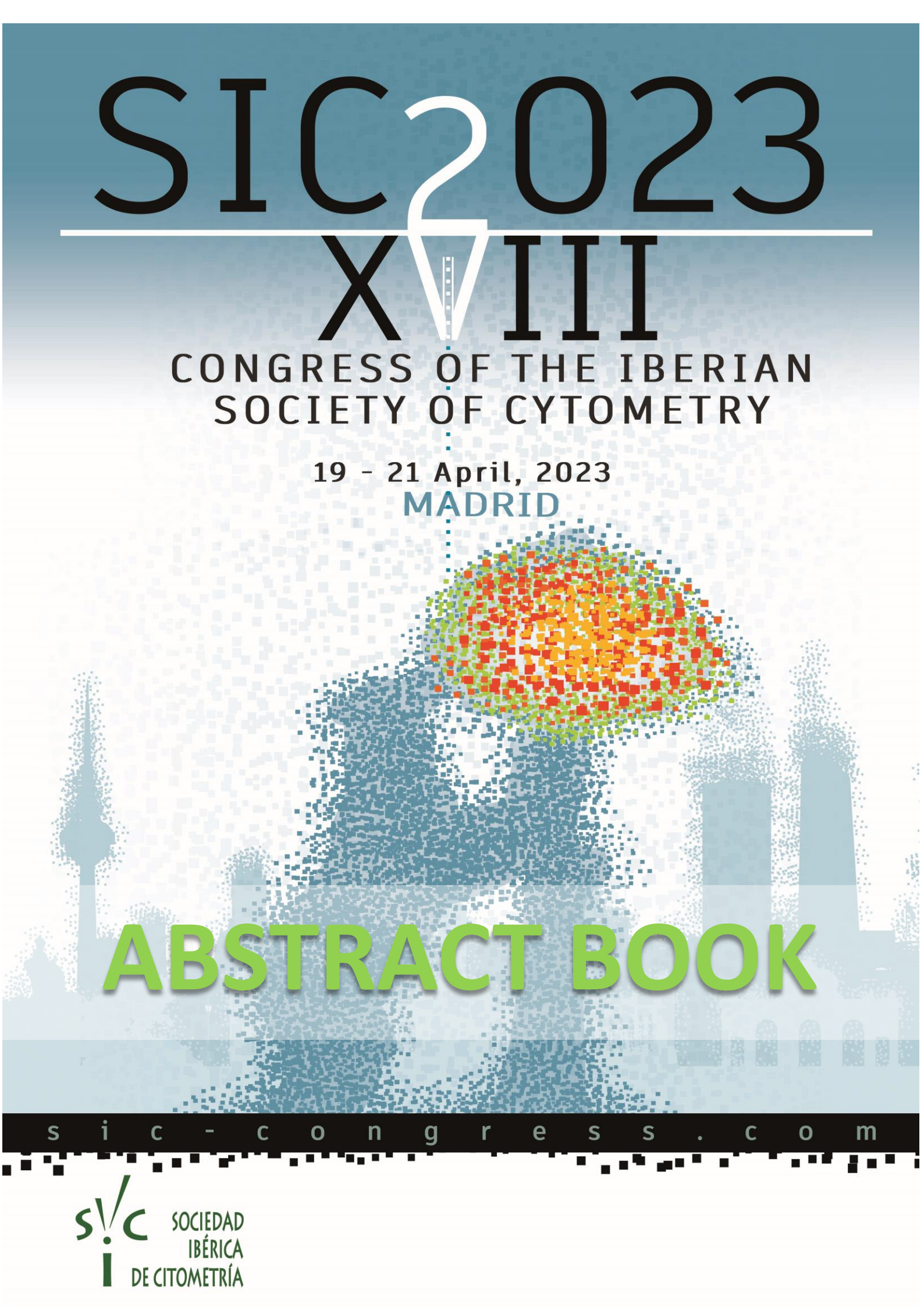

ABSTRACT BOOK

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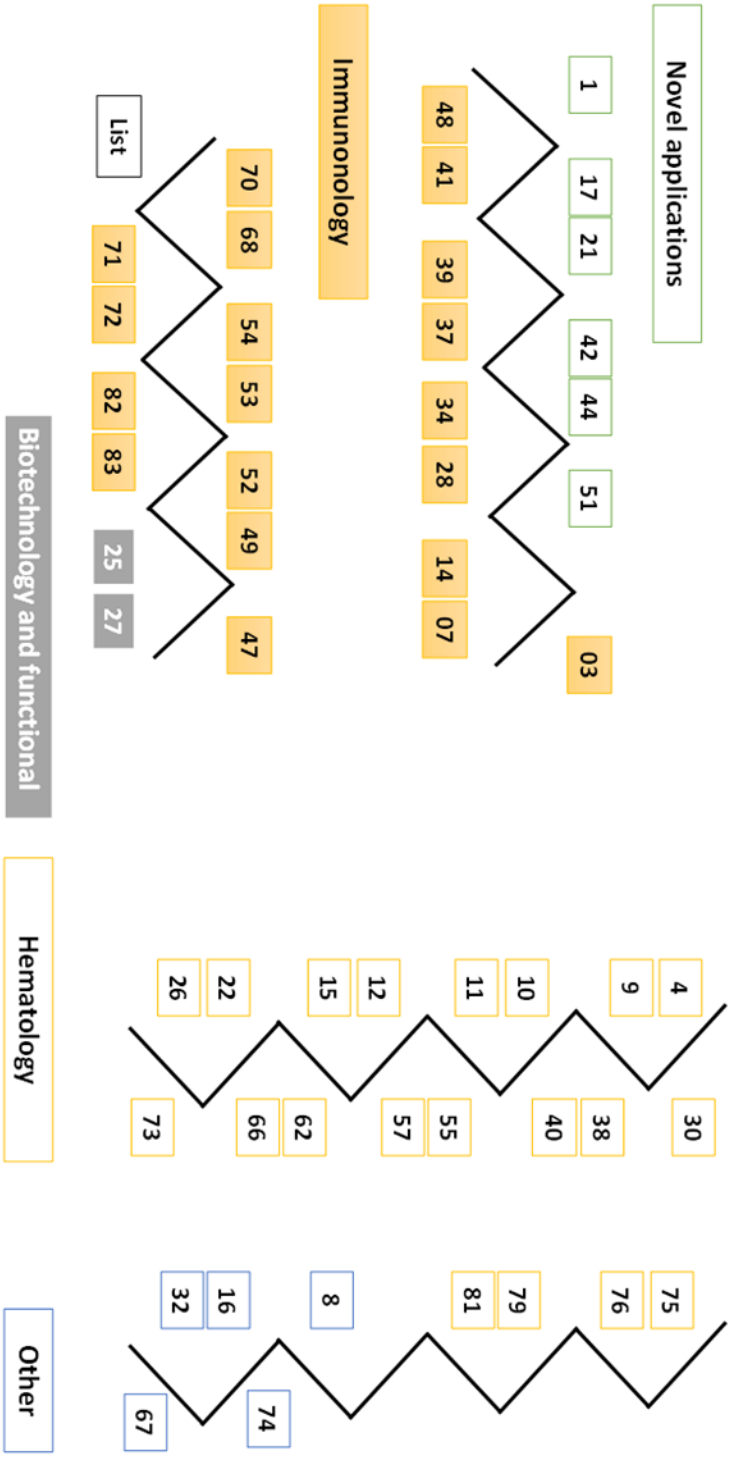
POSTER EXHIBITION HALL



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

AUTHORS

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

AUTHORS AFFILIATION

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

ABSTRACT

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

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

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

Results & Conclusion: FCI correctly diagnosed 453/522 samples (87.3%) suitable for FCI and cytology comparison. A final diagnosis of MPE was established in 182 samples: FCI identified 141/182 (77.4%) as compared to 94/182 (51.6%) by cytology ($p<0.0001$); most of them were epithelial-cell malignancies (117 vs. 81; $p<0.0001$). MPE not detected by cytology had significantly lower percentages of EpCAM+ cells as compared to cytology positive cases (0.02% vs. 1%; $p<0.0001$). Of note, 29/52 MPE (55.8%) only detected by FCI were new diagnosis of cancer, and 20 were lung tumours. Except for mesothelioma, FCI had better sensitivity than cytology for other lung subtypes, being particularly relevant squamous cell carcinoma (50% vs. 14.3%) and neuroendocrine tumours (86.7% vs. 33.3%).
In summary, this high-sensitivity FCI protocol significantly increases the diagnostic yield of pleural effusions, accelerating the diagnostic process in clinical practice. Even clinical laboratories with limited resources can easily implement the small panel designed to discard epithelial-cell malignancies.

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

AUTHORS

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

AUTHORS AFFILIATION

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

ABSTRACT

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

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

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

Results & Conclusion: Controls (n=35) and patient´s samples (n=96) showed higher levels of FcεRIα on basophils than on pDc (r2= 0.679). To normalise the wide range of expression, a ratio between basophils and T cells (negative control) MFI was calculated. Three levels of FcεRIα expression were observed: high (ratio ≥200), intermediate (ratio ≥100 and <200), and low (ratio <100). The distribution of FcεRIα levels was different in controls and pre-therapy samples (n=35) (p=0.015), with high levels in 20/35 patients (p<0.001). There was a weak positive correlation between serum IgE and FcεRIα expression (r2= 0.25). Anti-thyroid antibodies, basophils or pDc numbers could not predict FcεRIα levels. Both cells downregulated FcεRIα levels after first-therapy evaluation, but decline did not correlate with clinical response. Most patients with lower baseline levels (14/15; 93.3%) presented some kind of clinical improvement as compared to 13/18 patients (72%) with high levels (p=ns). In turn, 8/18 patients (44%) with high levels and 3/15 (20%) with lower levels needed updosing (p=ns).
In summary, while serial FC studies had no clinical interest, baseline data identified patients with low/intermediate FcεRIα levels who were more prone to respond to this omalizumab dosage. Larger studies are warranted to confirm these preliminary findings.

4. POSTER - ANGIOIMMUNOBLASTIC T-CELL LYMPHOMA WITH CD8 FOLLICULAR PHENOTYPE IN A PEDIATRIC PATIENT WITH TET2 MUTATION

AUTHORS

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

AUTHORS AFFILIATION

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

(2) Hospital De niños Ricardo Gutierrez. Buenos Aires. Argentina.

ABSTRACT

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

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

Results & Conclusion: Case report: A 10 years old (yo) girl born from non-consanguineous parents. At 8yo she was admitted to hospital due to prolonged fever, myalgia, periorbital edema, non-infectious and non-malignant lymphoproliferation with elevated inflammatory markers. Normal bone marrow (BM) biopsy. Autoinflammatory syndrome was suspected due to clinical and laboratory features. At 9yo she developed a new non-infectious lymphoproliferation event with fasciitis. BM biopsy showed myeloid hyperplasia with myelodysplastic focal changes. She received high dose corticosteroids with mild clinical and no laboratory parameters response. At 10yo she presented central nervous system (CNS) compromise with abnormal CNS CT and MRI. Biopsies: normal lymph node and liver with prominent CD8+Tcells infiltrate in bowel. Immunological laboratory findings showed: leukocytosis with CD8 lymphoproliferation, expanded CD3+TcRαβ+CD4-CD8- double-negative Tcells, low cTfh skewed towards th2 and low memory B cells. Molecular testing revealed a novel probably pathogenic de novo TET2 heterozygous non-sense variant (p.Arg1237*). Thereafter, she developed tonsillar hypertrophy and mediastinal widening. Tonsillar biopsy: AITL compatible. Flow cytometry: clonal Tcells: CD3+CD8+CD2+CD5+CD7+CXCR5+ICOS+/PD1+CD27+CD28+CD45RA-CCR7-HLADR+CD4-CD56-CD16-CD94-Perforin-GranzymeB-TRBC1- (tonsils and peripheral blood) with BM and cerebrospinal fluid compromise. She started chemotherapy with non-response and died during treatment. Our findings confirm a strong link between TET2 deficiency, immune dysregulation, and lymphomagenesis, with an extremely rare early onset angioimmunoblastic Tcell-lymphoma.

7. POSTER - CYTOKINES PROFILE DURING PLASMODIUM FALCIPARUM INFECTION IN A HUMANIZED MICE MODEL

AUTHORS
Cortés Gil, L.[1]

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

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

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

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

Results & Conclusion: We have set up a starting point of cytokines profile in the mouse model and we are able to determine the differences in this pattern in Host Directed Therapies assay, for preclinical studies in Malaria Drug Discovery. We have detected different inflammatory cytokines during the different stages of the protocol. The most expressed are MCP-1 expressed during engraftment process and IL6 during the infection and parasite exponential growth.
“The human biological samples were sourced ethically and their research use was in accord with the terms of the informed consents”
“All animal studies were ethically reviewed and carried out in accordance with European Directive 2010/63/EU and the GSK Policy on the Care, Welfare and Treatment of Animals.”

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

AUTHORS

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

AUTHORS AFFILIATION

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

ABSTRACT

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

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

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

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

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

AUTHORS

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

AUTHORS AFFILIATION

(1) Universitat de València

ABSTRACT

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

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

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

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

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

AUTHORS

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

AUTHORS AFFILIATION

(1) Universitat de València

ABSTRACT

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

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

Methods: We analysed intracellular as well as mitochondrial basal levels of ROS in leukocytes, neutrophils and lymphocytes and monocytes from aged mice (22 months) compared with aged mice supplemented with PE for four months. Blood from both aged groups was treated with an oxidant agent, tert-butyl hydroperoxide (tBHP) and was compared with non-oxidised blood. ROS levels were measured by flow cytometry using the intracellular fluorescence dye dihydrorhodamine123 (DHR123), 2,7-Dihydrodichlorofluorescein diacetate (DCF) and mitochondria peroxy yellow 1 (Mitophy1). The mean ± S.E.M. from four replicates was determined for each experimental group. All data were analysed using ANOVA with Graphpad Prism 8 software.

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

11. POSTER - HYPERTENSION INCREASES BASAL INTRACELLULAR PEROXYNITRITE LEVELS IN LEUKOCYTES OF MALE BUT NOT FEMALE RATS.

AUTHORS

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

AUTHORS AFFILIATION

(1) Department of Physiology, University of Valencia

(2) Flow Cytometry Unit, IIS INCLIVA, Fundación Investigación Hospital Clínico Valencia, 46010 Valencia, Spain.

ABSTRACT

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

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

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

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

12. POSTER - IS FLOW CYTOMETRY REALLY NOT USEFUL IN THE DIAGNOSIS OF HODGKIN LYMPHOMA?

AUTHORS
Álvarez Flores, M.[1], SOPEÑA CORVINOS, M.[2], GUILLÉN SANTOS, R.[2], CAVA VALENCIANO, F.[2]

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

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

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

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

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

Tube	FITC	PE	PerCP-Cy5.5	PE-Cy7	APC	APC-H7	V450	V500
1	**Kappa**	**Lambda**	**CD19**	**CD5**	**CD10**	**CD20**	**CD45**	HLA-DR*
2	**CD15**	**CD30**	**CD4**	**CD8**	**CD71**	**CD3**	**CD45**	KI67**
3	FMO	CD30	CD4	CD8	CD71	CD3	CD45	
4	CD15	FMO	CD4	CD8	CD71	CD3	CD45	
5	CD15	CD30	CD4	CD8	FMO	CD3	CD45	

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

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

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

AUTHORS

Kennedy Batalla, R.[1], Fernández Cooke, E.[2], Aracil Santos, J.[3], Saavedra, J.[4], García Salido, A.[5], Albendea Gómez, T.[6], Cano Nieto, M.[7], González, F.[4], Martín Puig, S.[8], Correa, R.[7]

AUTHORS AFFILIATION

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

ABSTRACT

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

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

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

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

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

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

AUTHORS

Barbosa Mendes, C.[1], Soares, R.[2], Santos, M.[3], Fonseca, S.[3], Oliveira, L.[3], Santos, F.[4], Martins Pereira, F.[4], Tente, D.[6], Lau, C.[4], Teixeira, M.[4]

AUTHORS AFFILIATION

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

ABSTRACT

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

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

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

Results & Conclusion: During the analyzed period, PMBCL was diagnosed in five patients, aged between 22 and 48 years, of which two were male and three females. The clinic at presentation was nonspecific. Among the initial symptoms there was a predominance of chest pain, cough, and absence of symptoms B. One patient presented with superior vena cava syndrome, in which thoracic CT showed an anterior mediastinal voluminous mass (>90mm), with invasion and stenosis of the local vascular structures. Flow cytometry immunophenotyping was performed in fine needle biopsy specimens from three of the five patients and in needle core biopsy of the remaining two patients. Mature phenotype B cells was identified. The expression of CD23 and CD30 was evaluated in four of the five samples (all of them were CD23 positive and two positive for CD30) and three samples were tested for CD200 (all positive), raising the suspected diagnosis of PMBCL, later confirmed by pathological study.
Flow cytometry immunophenotyping should be considered a viable and complementary approach to the pathological study, despite not being the gold-standard, since it increases initial diagnostic suspicion due to its rapid execution, interpretation and result that is important to guide the initial treatment.

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

AUTHORS

San Segundo, D.[1], COMINS BOO, A.[1], Pérez Pla, F.[2], Irure Ventura, J.[1], Martín Alonso, M.[3], López Hoyos, M.[1], Blanco Peris, L.[3]

AUTHORS AFFILIATION

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

ABSTRACT

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

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

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

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

17. POSTER - THE IMPORTANCE OF GOOD CONTROLS IN POLYCHROMATIC AND SPECTRAL FLOW CYTOMETRY

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

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

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

21. POSTER - BULK LYSIS PROCEDURES ALTER TARGET CELL POPULATION COUNTS

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

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

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

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

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

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

22. POSTER - MPDCP AN ENTITY ALREADY RECOGNIZED IN 5TH EDITION OF THE WHO

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

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

ABSTRACT
Background: An 80-years-old male arrived to the hospital with dyspnea and pruritic skin lesions. The physical exploration showed fever and eroded papules over his entire body surface. Our laboratory findings: Hemoglobin: 94g/L; White blood cell: 145*10^9/L; Platelets: 42*10^9/L; Absolute neutrophil count: 1.46*10^9/L; Blasts: 60%; Glomerular Filtrate: 20.84ml/min/1.73m2.
The diagnostic orientation was an acute leukemia with high risk of tumor lysis due to hyperleukocytosis and renal failure. The plan was a complete study of bone marrow aspirate, dermic biopsy and herpesvirus PCR.

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

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

Results & Conclusions: The results of the immunophenotype revealed 54,92% of Myeloid Blasts and additionally 11,72% of mature plasmacytoid dendritic cells (pDCs)(HLA-DR^{+}, CD123^{++}, CD4^{+}, CD36^{+}, CD38^{+}, CD56^{-}).
It is important to remember that there are two distinct types of neoplastic counterparts for pDCs; one is BPDCN, described in the WHO, and the other is MPDCP, only briefly mentioned and now recognized at the 5th edition of the WHO. The mature pDCs are characterized by HLA DR^{+} CD123^{++} CD56^{-} CD4^{+} CD38^{+} immunophenotype.
A cutaneous infiltrate composed of pDCs is frequently found in patients with MPDCP, like in this case.
The final diagnostic was an AML with pDCs with FLT3ITD and IDH2 mutations. Skin biopsy also described dendritic cells and the herpesvirus PCR was negative.

25. POSTER - VALIDATION OF CYTOCHECK SPACHIP® PH DETECTION KIT BY FLOW CYTOMETRY

AUTHORS

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

AUTHORS AFFILIATION

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

ABSTRACT

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

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

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

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

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

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

AUTHORS

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

AUTHORS AFFILIATION

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

ABSTRACT

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

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

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

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

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

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

AUTHORS

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

AUTHORS AFFILIATION

(1) Laboratory of Cytomics, Dpt. Biochemistry and Molecular Biology, University of Valencia,

(2) Cytometry Service, Central Research Unit (UCIM), Incliva Foundation-Valencia University, Valencia,

ABSTRACT

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

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

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

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

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

AUTHORS

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

AUTHORS AFFILIATION

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

ABSTRACT

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

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

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

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

30. POSTER - MONOCYTE-DERIVED SUBPOPULATIONS IN CLL PATIENTS COMPARED WITH HEALTHY DONORS.

AUTHORS

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

AUTHORS AFFILIATION

(1) Hematology Service. Hospital Ntra. Sra. Del Prado. (Talavera de la Reina),
(2) CIC. USAL.,
(3) Cytometry Service. Hematology. Hospital Ntra. Sra del Prado. Talavera de la Reina,

ABSTRACT

Background: Chronic lymphocytic leukaemia (CLL), a biologically and clinically heterogeneous condition, have an immunotolerant microenvironment that modulate CLL cells viability and survival.
In node biopsy samples and tissue models it has been observed that interactions between tumoral and monocyte-derived immune cells favours tumour cells growth. An elevated monocyte count in peripheral blood is related to accelerated disease progression and to time free of treatment.

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

Methods: We have analysed several monocyte-derived subpopulations in peripheral blood by multiparametric flow cytometry, as well as neutrophils, eosinophils, and lymphocytes in 17 CLL patients and 48 healthy controls.
Samples were stained and acquired according to Euroflow requirements in a FACSCANTO II cytometer. This samples had been analysed according published Euroflow strategy.

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

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

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

AUTHORS

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

AUTHORS AFFILIATION

(1) IPO-Porto, Porto,

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

ABSTRACT

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

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

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

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

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

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

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

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

AUTHORS

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

AUTHORS AFFILIATION

(1) CIEMAT/IIS-FJD/CIBERER,

ABSTRACT

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

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

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

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

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

AUTHORS

Torres Valle, A.[1], Neirinck, J.[2], De Arriba, S.[3], Aragon, L.[4], Silva, S.[5], Serrano, C.[6], Subirá, D.[7], Ruiz Mercado, M.[8], Gonzalez-Granado, L.[9], Marcos, M.[10], Inés, S.[3], Martins, C.[11], Hofmans, M.[12], De Vriendt, C.[12], Albarrán, B.[13], Hurtado, G.[14], Barez, A.[15], Arenas-Caro, P.[16], Madruga, I.[17], Martín, A.[18], Bastida, J.[18], Dávila, I.[19], Contreras-Sanfeliciano, T.[20], Jara, M.[21], Prieto, C.[22], Kerre, T.[23], Haerynck, F.[24], Sousa, A.[25], Prada, Á.[16], Bonroy, C.[12], Van Dongen, J.[26], Pérez-Andrés, M.[27], Orfao, A.[27]

AUTHORS AFFILIATION

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

(2) Department of Laboratory Medicine, Ghent University Hospital; Department of Diagnostic Sciences, Ghent University, Ghent, Belgium.,

(3) Servicio de Pediatría, Hospital Universitario de Salamanca, Salamanca, Spain.,

(4) Immunology Department, Donostia University Hospital, Osakidetza Basque Health Service, San Sebastián, Spain.,

(5) Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal.,

(6) Servicio de Inmunología, Fundación Jiménez Díaz, Madrid, Spain,

(7) Flow Cytometry Unit, Department of Hematology, Hospital Universitario de Guadalajara, Spain.,

(8) Hospital Costa del Sol, Hematologia y Hemoterapia, Marbella, Spain.,

(9) Immunodeficiencies Unit. Hospital 12 de octubre. Research Institute Hospital 12 octubre (i+12), Madrid, Spain.,

(10) Institute of Biomedical Research of Salamanca (IBSAL), Salamanca, Spain. Department of Internal Medicine, University Hospital of Salamanca, Salamanca, Spain,

(11) NOVA Medical School/Faculdade de Ciências Médicas Universidade Nova de Lisboa, Lisbon, Portugal,

(12) Department of Laboratory Medicine, Ghent University Hospital; Department of Diagnostic Sciences, Ghent University, Ghent, Belgium,

(13) Hematology, Complejo Asistencial Universitario, Palencia, Spain,

(14) Servicio de Hematología, Complejo Hospitalario de Navarra, Pamplona, España,

(15) Department of Hematology, Complejo Asistencial de Ávila, Spain,

(16) Immunology Department, Donostia University Hospital, Osakidetza Basque Health Service, San Sebastián, Spain,

(17) Servicio de Medicina Interna, Hospital Universitario de Salamanca, Spain,

(18) Servicio de Hematología, Hospital Universitario de Salamanca, Institute for Biomedical Research of Salamanca, Salamanca, Spain; Biomedical Research Networking Centre Consortium of Oncology (CIBERONC), Instituto de salud Carlos III, Madrid, Spain, (19) Servicio de Alergia, Hospital Universit

ABSTRACT

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

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

Methods: CD4+ T-cell subsets were analyzed in 53 CVID and 19 LOCID patients, in parallel with 146 healthy donors (4-88 years) using EuroFlow-based flow cytometry methods.

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

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

AUTHORS

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

AUTHORS AFFILIATION

(1) PhD student. Centro de Investigación del Cáncer. Salamanca,
(2) Translational and Clinical Research Program, Cancer Research Center (IBMCC, CSIC – University of Salamanca); Cytometry Service, NUCLEUS; Department of Medicine, University of Salamanca, Salamanca, Spain. IBSAL, Salamanca, Spain. CIBERONC ISCIII, Madrid, Spain.,
(3) Department of Haematology, University Hospital of Salamanca (HUS-IBSAL), CIBERONC, and Cancer Research Institute of Salamanca-IBMCC (CSIC-USAL University), 37007 Salamanca, Spain.,
(4) Department of Medicine, Cancer Research Centre (IBMCC, USAL-CSIC), Cytometry Service (NUCLEUS), University of Salamanca (USAL), Institute of Biomedical Research of Salamanca (IBSAL), Salamanca, Spain; Biomedical Research Networking Centre Consortium of Oncology (CIBERONC), Instituto de Salud Carlos III, Madrid, Spain

ABSTRACT

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

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

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

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

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

Results & Conclusions: Case#1 was referred for an expansion (1361 events/µL) of TαβCD3+CD4+CD7- cells with a TRBC1+/TRBC1- ratio of 0.58. In-depth flow-cytometry analysis revealed a suspicious (CD2++CD7-/+dimCD8-/+dimCD62L+) TαβCD4+ population with a CD27-CD45RA+cyGranzyme B+ terminal-effector phenotype (36.6% TRBC1+ and 63.4% TRBC1-). TCR gene rearrangement analysis of purified cell-fractions confirmed that they corresponded to non-related clonal populations, the TRBC1- population was found to have functionally rearranged TRBJ2 sequences.

In Case#2, a TαβCD3+CD8+CD5- population (986 events/µL) with a TRBC1+/TRBC1- ratio of 0.25 was detected in the screening tube. Both TRBC1+ (CD2+dimCD7-CD8+CD16+) and TRBC1- (CD2-/+dimCD7+CD8+dimCD16+) populations of terminal-effector cells were confirmed to correspond to different (unrelated) clones.

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

39. POSTER - ANALYSIS OF THE IMMUNOLOGICAL PROFILE OF SIX GOOD SYNDROME PATIENTS

AUTHORS

Torres Valle, A.[1], De Arriba, S.[2], Aragon, L.[3], Serrano, C.[4], Marcos, M.[5], Melero, J.[6], Arenas-Caro, P.[7], Jara, M.[8], Prieto, C.[9], Prada, Á.[7], Van Dongen, J.[10], Pérez de Andrés, M.[11], Orfao, A.[11]

AUTHORS AFFILIATION

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

(2) Servicio de Pediatría, Hospital Universitario de Salamanca, Salamanca, Spain.,

(3) Immunology Department, Donostia University Hospital, Osakidetza Basque Health Service, San Sebastián, Spain.,

(4) Servicio de Inmunología, Fundación Jiménez Díaz, Madrid, Spain,

(5) Institute of Biomedical Research of Salamanca (IBSAL), Salamanca, Spain. Department of Internal Medicine, University Hospital of Salamanca, Salamanca, Spain,

(6) Servicio de Inmunología y Genética, Hospital Infanta Cristina, Complejo Hospitalario Universitario de Badajoz, Badajoz, Spain.,

(7) Immunology Department, Donostia University Hospital, Osakidetza Basque Health Service, San Sebastián, Spain,

(8) DNA Sequencing Service (NUCLEUS), University of Salamanca, Salamanca, Spain. Institute of Biomedical Research of Salamanca (IBSAL), Salamanca, Spain; Biomedical Research Networking Centre Consortium of Oncology (CIBERONC), number CB16/12/00400, Instituto de Salud Carlos III, Madrid, Spain,

(9) Bioinformatics service (NUCLEUS), University of Salamanca, Salamanca, Spain,

(10) Department of Medicine, Cancer Research Centre (IBMCC, USAL-CSIC), Cytometry Service (NUCLEUS), University of Salamanca (USAL), Institute of Biomedical Research of Salamanca (IBSAL), Salamanca, Spain; Biomedical Research Networking Centre Consortium of Oncology (CIBERONC), Instituto de Salud Carlos III, Madrid, Spain; Department of Immunology, Leiden University Medical Center,

(11) Department of Medicine, Cancer Research Centre (IBMCC, USAL-CSIC), Cytometry Service (NUCLEUS), University of Salamanca (USAL), Institute of Biomedical Research of Salamanca (IBSAL), Salamanca, Spain; Biomedical Research Networking Centre Consortium of Oncology (CIBERONC), Instituto de Salud Carlos III, Madrid, Spain

ABSTRACT

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

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

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

Results & Conclusions: All patients consistently presented with lack of B-cells (<0.02 cells/µL). In addition, alterations were also found in other immune subsets including significantly reduced counts of total CD4+

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

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

AUTHORS

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

AUTHORS AFFILIATION

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

ABSTRACT

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

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

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

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

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

AUTHORS

Fuentes-Herrero, B.[1], Oliva-Ariza, G.[1], Carbonell, C.[2], Lecrevisse,, Q.[3], Perez-Pons, A.[1], Torres-Valle, A.[1], Pozo, J.[1], Martín-Oterino, J.[4], González-López, Ó.[1], López-Bernús, A.[5], Bernal-Ribes, M.[3], Belhassen-García, M.[5], Pérez-Escurza, O.[1], Pérez de Andrés, M.[6], Vazquez, L.[7], Hernández-Pérez, G.[8], García Palomo, F.[9], Leoz, P.[10], Costa-Alba, P.[11], Pérez-Losada, E.[12], Yeguas, A.[10], Santos Sánchez, M.[1], García-Blázquez, M.[13], Morán-Plata, F.[1], Damasceno, D.[14], Botafogo, V.[1], Muñoz-García, N.[1], Fluxa, R.[15], Contreras-Sanfeliciano, T.[16], Marcos, M.[4], Almeida, J.[14], Orfao, A.[6]

AUTHORS AFFILIATION

(1) Translational and Clinical Research Program, Cancer Research Center (IBMCC, CSIC – University of Salamanca); Cytometry Service, NUCLEUS; Department of Medicine, University of Salamanca, Salamanca, Spain. Institute of Biomedical Research of Salamanca (IBSAL), Salamanca, Spain,

(2) Institute of Biomedical Research of Salamanca (IBSAL), Salamanca, Spain. Department of Internal Medicine, University Hospital of Salamanca, Salamanca, Spain. Institute of Biomedical Research of Salamanca (IBSAL), Salamanca, Spain,

(3) Translational and Clinical Research Program, Cancer Research Center (IBMCC, CSIC – University of Salamanca); Cytometry Service, NUCLEUS; Department of Medicine, University of Salamanca, Salamanca, Spain,

(4) Institute of Biomedical Research of Salamanca (IBSAL), Salamanca, Spain. Department of Internal Medicine, University Hospital of Salamanca, Salamanca, Spain,

(5) Institute of Biomedical Research of Salamanca (IBSAL), Salamanca, Spain. Department of Internal Medicine, University Hospital of Salamanca, Salamanca, Spain.Department of Infectious Diseases, University Hospital of Salamanca, Centro de Investigación de Enfermedades Tropicales de la Universidad de Salamanca (CIETUS), Salamanca, Spain,

(6) Department of Medicine, Cancer Research Centre (IBMCC, USAL-CSIC), Cytometry Service (NUCLEUS), University of Salamanca (USAL), Institute of Biomedical Research of Salamanca (IBSAL), Salamanca, Spain; Biomedical Research Networking Centre Consortium of Oncology (CIBERONC), Instituto de Salud Carlos III, Madrid, Spain,

(7) Institute of Biomedical Research of Salamanca (IBSAL), Salamanca, Spain.Department of Hematology, University Hospital of Salamanca, Salamanca, Spain,

(8) Department of Internal Medicine, University Hospital of Salamanca, Salamanca, Spain,

(9) Spanish National DNA Bank Carlos III, University of Salamanca, Salamanca, Spain,

(10) Institute of Biomedical Research of Salamanca (IBSAL), Salamanca, Spain. Department of Hematology, University Hospital of Salamanca, Salamanc

ABSTRACT

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

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

Methods: 802 samples from 343 patients (median 57 years -y-, from 18-99y) were analyzed, including 23 cases (n=132 samples) studied every 24h-48h during the acute phase. The distribution of cell-populations was analyzed by flow-cytometry; plasma levels of anti-virus N-protein antibodies were determined by ELISA. Results were relativized from the day of symptoms onset (d0) and normalized by age with healthy donors (n=928). SARS-CoV-2 viral load was quantified in plasma by RT-PCR (n=303 samples).

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

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

AUTHORS

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

AUTHORS AFFILIATION

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

ABSTRACT

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

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

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

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

44. POSTER - T CELL CLONALITY EVALUATION IN SCREENING OF SAMPLES SUSPECT LYMPHOMAS BY FLOW CYTOMETRY WITH TRBC1

AUTHORS

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

AUTHORS AFFILIATION

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

ABSTRACT

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

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

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

Results & Conclusions: We observed that the TRBC1+/- ratio in normal samples was between 0.33 and 1.1 for TαβCD4+ cells and between 0.22 and 1.0 for TαβCD8+ cells. Monophasic TRBC1 expression was identified in 18 samples from patients with T-cell malignancies. Inclusion of TRBC1 in routine suspected lymphoma samples improves the detection of T cell clones compared to our established CD4/CD8 ratio. The results of these analyses correlate with histopathological findings.

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

AUTHORS

Rybakowska, P.[1], Van Gassen, S.[2], Barturen, G.[3], Pérez Sánchez, C.[4], Ibáñez-Costa, A.[4], Varela, N.[3], Ortega Castro, R.[4], Fernández-Roldán, C.[5], Jiménez-Moleón, I.[6], Ortego, N.[5], Raya, E.[6], Aguilar Quesada, R.[7], López-Pedrera, C.[8], Collantes, E.[8], Saeys, Y.[9], Alarcón-Riquelme, M.[10], Marañón, C.[11]

AUTHORS AFFILIATION

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

ABSTRACT

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

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

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

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

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

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

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

AUTHORS

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

AUTHORS AFFILIATION

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

ABSTRACT

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

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

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

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

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

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

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

AUTHORS

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

AUTHORS AFFILIATION

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

ABSTRACT

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

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

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

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

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

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

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

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

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

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

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

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

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

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

AUTHORS

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

AUTHORS AFFILIATION

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

ABSTRACT

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

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

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

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

protein showed significantly different median of 0.07% vs 0.66% BM cPC ($p<0.001$), and of blood CcPC ($p=0.01$). Overall, our results show a close relationship between the serum M-component concentration and the percentage of cPC in BM and blood, which can help optimize the diagnostic approach of early vs advanced stage MG.

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

AUTHORS

Pereiro Rodríguez, A.[1], Mohamed Mohamed, K.[2], Guerra Galán, T.[2], Palacios Ortega, M.[2], Mansilla Ruíz, M.[2], Villegas Mendiola, Á.[2], García Bravo, L.[2], Guzmán Fulgencio, M.[3], Fernández Arquero, M.[4], Sánchez Ramón, S.[5]

AUTHORS AFFILIATION

(1) Residente de Inmunología en Hospital Clínico San Carlos.,
(2) Residente en Inmunología. Hospital Clínico San Carlos.,
(3) Adjunta Servicio de Inmunología Hospital Clínico San Carlos,
(4) Adjunto Servicio de Inmunología Hospital Clínico San Carlos,
(5) Jefe Servicio de Inmunología. Hospital Clínico San Carlos

ABSTRACT

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

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

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

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

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

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

AUTHORS

Comins Boo, A.[1], Fernández Cabero, J.[1], Lasa Teja, C.[2], Loricera, J.[2], Corrales, A.[2], Bejerano, C.[2], Martínez Taboada, V.[2], San Segundo, D.[3], Blanco, R.[2], López-Hoyos, M.[3]

AUTHORS AFFILIATION

(1) University Hospital Marqués de Valdecilla. Santander,
(2) Rheumatologist. University Hospital Marqués de Valdecilla. Santander,
(3) Immunologist. University Hospital Marqués de Valdecilla. Santander

ABSTRACT

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

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

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

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

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

55. POSTER - BLASTIC PLASMACYTOID DENDRITIC CELL NEOPLASM: A RARE DIAGNOSIS

AUTHORS

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

AUTHORS AFFILIATION

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

ABSTRACT

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

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

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

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

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

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

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

AUTHORS

Ballester González, R.[1], Rodríguez Martín, E.[2], Roy Ariño, G.[3], Manterola Navarro, E.[4], Ciancas, E.[5], Villar Guimerans, L.[2], Roldán Santiago, E.[2]

AUTHORS AFFILIATION

(1) Resident in Immunology. Ramón y Cajal University Hospital. Madrid,
(2) PhD. Immunologist. Hospital Universitario Ramón y Cajal. Madrid,
(3) MD-PhD. Immunologist. Hospital Universitario Ramón y Cajal. Madrid,
(4) Resident in immunology. Hospital Universitario Ramón y Cajal. Madrid,
(5) MD. Ophthalmologist. Hospital Universitario Ramón y Cajal. Madrid

ABSTRACT

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

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

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

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

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

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

AUTHORS

Jimenez-Leon, M.[1], Delgado-Serrano, J.[2], Sanoja-Flores, L.[3], Ruiz, V.[3], Lopez-Corral, L.[4], Valcarcel, D.[5], Ferra-Coll, C.[6], Caballero-Velázquez, T.[2], Pérez-Simón, J.[2]

AUTHORS AFFILIATION

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

ABSTRACT

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

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

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

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

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

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

AUTHORS

Fernández, G.[1], Pérez De Camino Gaisse, B.[2], Soto, C.[2], Salgado, R.[2], Izquierdo Barba, I.[3], Serrano Del Castillo, C.[4], Serrano, J.[5], Llamas Sillero, P.[6], González, V.[7], Serrano López, J.[8]

AUTHORS AFFILIATION

(1) Aptus Biotech S.L.,
(2) Hematology Department, Hospital Universitario Fundación Jiménez Díaz, UAM, Madrid, Spain,
(3) Departamento de Química en ciencias Farmacéuticas, Facultad de Farmacia, Universidad Complutense de Madrid, Instituto de Investigación Sanitaria Hospital 12 de Octubre i+12, Plaza Ramón y Cajal s/n Madrid, 28040, Spain.; CIBER de Bioingeniería Biomateriales y Nanomedicina CIBER-BBN, Madrid, 28040, Spain.,
(4) Immunology department, Hospital Universitario Fundación Jiménez Díaz, UAM, Madrid, Spain,
(5) Hematology Department, Reina Sofía University Hospital/Maimonides Biomedical Research Institute of Córdoba (IMIBIC)/University of Córdoba, Spain.,
(6) Hematology Department, Hospital Universitario Fundación Jiménez Díaz, UAM, Madrid, Spain; 8Experimental Hematology Lab, IIS-Fundación Jiménez Díaz, UAM, Madrid, Spain.,
(7) Grupo de Aptámeros, departamento de Bioquímica-Investigación, IRYCIS-Hospital Ramón y Cajal, 28034 Madrid, Spain.,
(8) Experimental Hematology Lab, IIS-Fundación Jiménez Díaz, UAM, Madrid, Spain

ABSTRACT

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

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

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

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

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

AUTHORS

Marquina Rodríguez, Á.[1], Ortega, M.[2], García-Lorena, P.[3], Barroso-García, G.[4], Arandilla, A.[4], González López, M.[5], Clemente, D.[2], Vila-del Sol, V.[5]

AUTHORS AFFILIATION

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

ABSTRACT

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

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

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

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

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

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

AUTHORS AFFILIATION
(1) Centro Nacional de Investigaciones Oncologicas, Madrid

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

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

Methods: Using a series of animal models combined with comprehensive analyses of patient tissue biopsies and datasets, we have previously identified the growth factor MIDKINE (MDK) as a new driver of pre-metastatic niches in melanoma.

Results & Conclusion: Moreover, our laboratory has identified reported MDK as an immune suppressor. Analyzing single-cell RNA sequencing data from bone marrow cells, MDK is particularly expressed in a subtype of reticular cells (Cxcl12-positive). These cells do not express the same levels of pleiotrophin (PTN), a protein that is highly homolog to MDK. This distinct expression of MDK and PTN may offer the possibility of differing roles in melanoma progression and modulation of immunomodulatory signals.

70. POSTER - ANTITUMORAL ROLES OF MIDKINE VIA B CELLS

AUTHORS

Juan Larrea, N.[1]

AUTHORS AFFILIATION

(1) Centro Nacional de Investigaciones Oncologicas. Madrid

ABSTRACT

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

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

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

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

71. POSTER - TREATMENT SCHEME AFFECTS LYMPHOCYTE SUBSETS IN SLE PATIENTS.

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

AUTHORS AFFILIATION
(1) H.U. Fundación Jiménez Díaz. Madrid,

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

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

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

Results & Conclusion: We studied 39 patients (mean age 49±13 years, 95% women) with SLE in treatment with: corticoids (CS, in any scheme) 26%; immunomodulators (IMD, in any scheme) 54%; rituximab (RTX) 8%; and others,13%. Median immunoglobulin levels, B, T and NK lymphocytes were within age reference values. We observed differences between patients treated with IMD and patients treated with CS in absolute leukocyte count (4775 cel/ul vs 7507 cel/ul, p=0.0038), monocytes (434 cel/ul vs 692 cel/ul, p=0.0014) and lymphocytes (1429 cel/ul vs 2538 cel/ul, p=0.0029).
In the analysis of the different lymphocyte subsets, we found that 50% of the patients present NK lymphopenia without any differences in median NK lymphocytes between groups. Patients treated with IMD had a lower absolute T cell count compared to CS treated patients (1180 cel/ul vs 2181 cel/ul, p=0.0037) due to CD4 lymphocytes (640 cel/ul vs 1282 cel/ul, p=0.0003).

Regarding B cells, we could not find any differences between groups, nevertheless, 50% of our patients present more than 2% of PC. There were not any differences in serum immunoglobulins between any group.
There are no significant changes in SLE patients treated with different therapeutic regimens. However, NK lymphopenia and presence of PC are common in some patients.

72. POSTER - LYMPHOCYTE SUBSETS IN A IGA DEFICIENCY COHORT THAT INCLUDES PATIENTS WITH CELIAC DISEASE

AUTHORS

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

AUTHORS AFFILIATION

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

ABSTRACT

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

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

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

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

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

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

AUTHORS

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

AUTHORS AFFILIATION

(1) CIEMAT-CIBERER / IIS-FJD,

(2) IMIM

ABSTRACT

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

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

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

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

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

AUTHORS

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

AUTHORS AFFILIATION

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

ABSTRACT

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

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

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

Results & Conclusion: Our results have shown a diminished activation of platelets in our FA cohort in comparison with healthy donors. Both signal of PAC-1 and Fibrinogen-Alexa488 decreased after stimulation with PMA while no differences were observed after PAR-1 and ADP stimulus.
In summary, flow cytometry is a feasible strategy to analyze platelet function in FA patients and will contribute to understand the pathophysiology of the thrombocytopenia in FA and other bone marrow failure syndromes.

75. POSTER - CONGENITAL LEUKEMIA OR TRANSIENT ABNORMAL MYELOPOIESIS? A CASE REPORT

AUTHORS

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

AUTHORS AFFILIATION

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

ABSTRACT

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

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

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

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

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

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

AUTHORS

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

AUTHORS AFFILIATION

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

ABSTRACT

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

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

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

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

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

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

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

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

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

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

Results & Conclusion: A total of 140 BM samples from 91 patients (59 male, 32 female; median age 68) were included. 10 patients had high-risk disease. In our cohort, 65% (59) of patients were in VGPR or better. All patients had at least 1 MRD evaluation, 40% (36) had 2, and 14% (13) had 3 or more. The sensitivity threshold was 10-5 in 50% of analyzed samples. In the remaining, this sensitivity level was not attained, mostly due to insufficient sample volume, low cellularity and/or hemodilution. MRD negativity rate overall was 30%. Noteworthy, 40% of high-risk patients achieved MRD negativity. Of the patients that had at least 2 evaluations, 54% maintained MRD negativity throughout the assessment period. Interestingly, after a median follow-up of 48 months of the 3 patients with MRD evaluations at least 12 months apart, all had sustained MRD negativity.
Our experience highlights that effective monitoring of MRD by NGF is feasible and can be incorporated as a biomarker of response in routine patient care. Real-world studies with long-term follow-up MRD data can provide critical insights to maximize treatment benefits.

81. POSTER - USEFULNESS OF IMMUNOPHENOTYPING IN THE EARLY DIAGNOSIS OF POST-TRANSPLANT LYMPHOPROLIFERATIVE DISEASE (PTLD): A CASE REPORT

AUTHORS

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

AUTHORS AFFILIATION

(1) Complexo Hospitalario Universitario of Santiago de Compostela

ABSTRACT

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

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

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

Results & Conclusion: We present a 58-year-old patient diagnosed in April 2022 with IgA lambda plasma cell leukaemia (15% of plasma cells in periheral blood (PB)), presenting 23.63% of atypical plasma cells in bone marrow (BM) at diagnosis with the following immunophenotype: CD38+, CD138+, CD19-, CD56 +lo/+, CD45-, CD117-, CD27+lo, CD81+. She received treatment with chemotherapy first, and an alloHSCT was infused on 25/10. She was admitted due to fever on 30/12. Bone marrow immunophenotyping was performed, finding 0.13% in BM of atypical plasma cells with an immunophenotype different from that of the diagnosis, and 2.5% in PB (cytoplasmic kappa monoclonality, CD38+, CD138+, CD19+, CD56-, CD45+, CD117-, CD27+, CD81+), with undetectable minimal residual disease. FISH concluded that the origin of the clone came from the donor cells. This led to a diagnosis of PTLD and targeted treatment could be initiated. Use of immunophenotyping is helpful in the diagnosis of PTLD, as it offers the following advantages:
- Speed in obtaining the diagnosis, compared to histopathology.
- Characterisation of the abnormal phenotype, which may not be differentiated in the histological study.
This could reduce the mortality associated with PTLD.

82. POSTER - USING FLOW CYTOMETRY TO UNCOVER CIRCULATING AND PERITONEAL NK CELL PROFILES IN ENDOMETRIOTIC WOMEN.

AUTHORS

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

AUTHORS AFFILIATION

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

ABSTRACT

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

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

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

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

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

AUTHORS

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

AUTHORS AFFILIATION

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

ABSTRACT

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

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

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

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

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POSTER EXHIBITION HALL