

TCS 2024

The Cytometry Society-India



Theme
Unraveling New Horizons of Cytometry

17-20 Oct 2024

16th
Annual Conference
& Workshops

e-Souvenir



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Organizing Secretary-Basic Science
Dr. Vainav Patel



16th Annual Conference & Workshops

TCS 2024

Speakers and Faculty



Alberto Orfao
(Spain)



Aaron Tynnik
(USA)



Brent Wood
(USA)



Jahnavi Aluri
(USA)



Kimberly Gilmour
(USA)



Orianne Wagner
Ballon (France)



Roshini
Abraham (USA)



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(Singapore)



Mubin Tarannum
(USA)



A Nisar



A Handoo



A Bhat



A Chaturvedi



A Sing



A Gupta



B Bagal



D Kumar



D Panda



Ganesh Kumar



H Krishnamurthy



K Bommannan



K Rehman



M Sachdeva



N Mallik



N Sachdeva



N Gupta



N Jindal



R Pandey



S Gupta



S Epari



V Chatterji

16th Annual Conference & Workshops TCS 2024

Message from Dr Urmi Chatterji, President of the Cytometry Society, India

The TCS is celebrating 16 years of its inception at ACTREC, Mumbai. Since its inception, the society has endeavored to advocate the applications of flow cytometry in basic research and in clinical diagnosis. Through the years, flow cytometry has become an integral and indispensable technique for researchers all over the world. The technology has improved immensely through the years and science has progressed in terms of speed, accuracy and innovations.

I wish the Organizing Team of this workshop and conference the very best and hope everyone who attends the same an incredible experience of learning and interaction.

Best wishes!!

Urmi Chatterji
Professor, University of Calcutta



16th Annual Conference & Workshops TCS 2024

Message from the Dr. Vainav Patel and organizing team

Hello there Flow'rs, FACS'rs and every cytometrist in between! A warm welcome to The Cytometry Society of India 16th Annual Conference and Workshops jointly hosted by ACTREC (TMC), ICMR-NIRRCH and ICMR-NIIH. We are back in the maximum city after 9 years with yet another scientific and technological feast that we hope will satisfy both newer entrants to our field as well as provide a thorough update to our more experienced community.

This year, spread across 3 sites, more than 10 workshops covering both clinical and basic sciences such as hematological oncology, immunology, image enabled sorting and high dimensional data driven discovery will be conducted concurrently on October 17th and 18th, 2024 for the benefit of more than 150 registered participants and participating faculty.

As usual, this will be immediately followed by our Annual Meeting on October 19th and 20th in which we are very happy to announce the participation of more than 250 delegates, international and national faculty with 6 key note presentations, 12 invited talks and most importantly – 10 young investigator oral presentations as well as almost 100 poster presentations. In our continuing commitment to fostering and inspiring the future generation of basic scientists and clinical researchers a total of 10 travel awards and generous prizes for both oral and poster presentations have been allocated.

A highlight of this year's meeting will include the best published paper award presentations in both basic and clinical categories as well as the honouring of our stalwart member, Dr Amar Dasgupta, with The TCS 2024 Oration Award.

Of course, without the invaluable support of our technology partners and sponsors, we would never have been able to organize this event in its current form or scale. We gratefully acknowledge the support of BD Biosciences, Beckman Coulter, Cytek and others.

Finally, we welcome you all again and wish you a wonderful time in Mumbai!



Dr. Vainav Patel
Head of Viral Immunopathogenesis, NIRRCH, Mumbai



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INDEX

1. Program at a glance	6-13
2. Bio-sketch of speakers and summary of talks	14-41
3. Oral Abstracts (Clinical)	42-51
4. Oral Abstracts (Basic)	52-65
5. Poster Abstracts (Clinical)	66-145
6. Poster Abstracts (Basic)	146-190
7. Sponsor	191

16th Annual Conference & Workshops

TCS 2024

Conference Schedule: 19th October 2024

ACTREC, Tata Memorial Centre, Navi Mumbai

Time	Topics
	Hall-KS

8`30-9`10	Registration & Inauguration	
9`15-9`55	Internal and External Cellular dynamics reveal insights into differential infectious Disease Severity.....	R Pandey
10`00-10`45	Circulating tumor cells (CTC) in PCN, ALL & other HLN.....	A Orfao
10`45-11`00	BD Scientific lecture	A Tyznik
11`00-11`30	Breakfast	

	Hall-KS	Hall-PS	Hall-RRU
11`30-11`50	Markers predicting underlying genetics in AL.... S Rajpal	Correcting misdiagnosis: T-NHL P Tembhare	Stem cells and Cancer ... U Chatterjee

11`55-12`15	Markers predicting outcome in B-ALL K Bommannan	T-cell Clonality: TRBC1..... K Rahman	Cellular Therapy in ovarian cancer..... T Mubin
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12`20-13`00	ALL MRD in CAR-T therapy.... B Wood	Immunophenotyping in Systemic Mastocytosis A. Orfao	Making CAR T cells in India for India.... A Nisar
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13`00-13`10	Scientific Corporate lecture	Scientific Corporate lecture	Scientific Corporate lecture
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13`10-14`10	Lunch Break	Hall-KS
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14`15-14`45	Defining Lineage in acute leukemia – WHO 5 th Edition.... B Wood	
14`50-15`20	Engineered exosomes from Tregs: novel therapy for Type-1 Diabetes	N Sachdeva
15`25-15`40	BC Scientific lecture	V Ruocco
15`40-16`20	TCS Best Published Paper Award Felicitations	
16`20-17`00	Tea/Coffee Break	

	Hall-KS	Hall-PS
17`00-17`40	Oral presentations: Clinical	Oral presentations: Basic
17`45-19`00	Poster evaluation / EC meeting	

19`30 onwards	Welcome Dinner
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16th Annual Conference & Workshops TCS 2024

Conference Schedule: 20th October 2024

ACTREC, Tata Memorial Centre, Navi Mumbai

Time	Topics
	Hall-KS
8'00-8'35	Navigating T-cell landscapes with advanced flow cytometry.. N Gupta
8'40-9'20	Monocyte/macrophage/PDC: Implications for the diagnosis and classification of AML A Orfao
9'25-10'00	Innate immunity in tuberculosis pathogenesis.... D Kumar
10'00-10'25	Breakfast
10'30-11'20	TCS Oration Award Ceremony
	Hall-KS
11'30-12'00	Interesting clinical case presentation Moderated by S Gujral & G Chatterjee Dia Mansukhani Vasudha Kaul Sananda Kumar Sonia Nagyal Pratishttha Madan Sabina Langer Asish Rath Sukriti Thakur Sanjoli Chugh Mona Singh Ananthvikas
12'00-12'30	Primary immunodeficiency..... M Madkaikar
12'30-13'00	Cell free therapeutic approach for impaired wound healing... A Bhat Intertwined paths of standardization, automation and AI in flow cytometry P Jain
13'00- 14'00	General Body meeting, Best Paper Awards, Travel awards & Vote of Thanks
14'00 onwards	Lunch

16th Annual Conference & Workshops TCS 2024

Workshops: 17th October 2024

ACTREC, Tata Memorial Centre, Navi Mumbai

Registration: 08`00~9`00

Common Lectures: Hall-KS

9'00-9'30	Basics of Flow Cytometry	P Tembhare
9'30-10'00	Deciding Panel: Factors to consider	PG Subramanian

Breakfast

Workshop-1a: Approach to ALL & ALL MRD ~ Day-1	(Hall-KS)
11'00-11'30 FCM in Acute Leukemia & lineage assignment	S Gupta
11'30-12'00 Baseline Risk-stratification in ALL	N Mallik
12'00-13'00 Live analysis of ALL cases	A Karla, A Bibi
14'00-14'25 FCM approach of B-ALL MRD	D Panda
14'30-14'55 High sensitivity & Post antiCD19 therapy B-MRD	G Chatterjee
15'00-15'30 BMRD live case analysis	P Dhende, M Singh

Tea/Coffee Break

16'00-16'30 FCM approach to T-ALL MRD	A Gupta
16'35-17'00 T-MRD live case analysis	G Chatterjee

Workshop-2: Approach to CMML MDS, MPN	(HALL-PS)
11'00-11'40 Immunophenotypic approach to CMML	O Wagner-Ballon
11'40-13'00 CMML live case analysis	O Wagner-Ballon
14'00-15'00 Normal Myeloid/Erythroid maturation, stem cell abnormalities in MDS and JMML	A Orfao
15'00-15'30 FCM in JMML and other MPN: TMC experience	P Tembhare
Tea/Coffee Break	
16'00-16'30 Immunophenotypic approach to MDS diagnosis	B Wood
16'35-17'00 MDS cases – TMC	P Tembhare

16th Annual Conference & Workshops TCS 2024

Workshops: 17th October 2024

ACTREC, Tata Memorial Centre, Navi Mumbai

Workshop-3a: Approach to B-CLPD & MM ~ Day-1	(Hall-PTC)
11'00-11'30 Immunophenotypic approach to B-CLPD	Ganesh KV
11'30-12'30 New updates in B-CLPD and live cases	A Orfao
12'35-13'00 FCM in marrow Involvement by BNHL	A Singh
14'00-14'25 Clinical value of BM involvement in NHL	B Bagal
14'30-15'30 B-CLPD Live cases analysis	S Sharma, K. Galani K Ghodke, A Vazifdar S Harankhedkar, M Kar
Tea/Coffee Break	
16'00-16'30 FCM Approach to Plasma Cell Disorders	MUS Sachdeva
16'35-17'00 MM Post-therapy assessment & MRD monitoring	S Java, B Bagal MUS Sachdeva

Workshop-4: Spectral Flow Cytometry	(HALL-CCE)
11'00-11'40 Introduction to Spectral Flow Cytometry	Aaron Tynznik (USA)
11'45-12'30 Panel Designing for spectral flow cytometry	Aaron Tynznik
12'30-13'00 Setting up Reference controls & Unmixing	Aaron Tynznik
14'00-14'30 Introduction to Software Analysis	S Basu
14'30-15'30 Live cases: Spectral FCM	S Ghogale/J Patil
Tea/Coffee Break	

Scientific Corporate session: BD Biosciences	(HALL-KS)
17'00-17'40 What's New!!	BD India Team

17'30-18'00 Evening - Snacks & Tea/Coffee

16th Annual Conference & Workshops TCS 2024

Workshops: 17th October 2024

ICMR NIIH & ICMR NIRCH, Mumbai

Time 9`00~12`30

Common Lectures (NIRCH)

- | | |
|---|-----------------|
| ... Basics of Flow Cytometry | H Krishnamurthy |
| ... Application of Flowcytometry and Discussion | P Jain |
| ... Panel Designing | S Walke |

Time 13`30~18`00

Workshop-6: High Dimensional Data-Driven Discovery (NIRCH)

- ... 30 colour panel acquisition on High Dimensional Cytometer BD Symphony A3
- ... Tutorial on FlowJo Conventional and Unsupervised high dimensional data analysis

Workshop-7: Conventional & Image Enabled Sorting (NIRCH)

- ... Hands-on & Tutorial: Conventional and Spectral Sorting

Workshop-8: Inborn Errors of Immunity (IEI) (NIIH)

- | | |
|--|------------------|
| ... Overview of IEIs | M Madkaikar |
| ... Diagnostic approach to Combined Immunodeficiency | |
| Hands-on processing in extended-LSS & Analysis | M Gupta/S Shinde |
| ... Diagnostic approach to Phagocytic defects & | |
| Hands-on NBT/ DHR and Analysis | U Bargir/A Dalvi |
| ... Complete automation of PIDOT | BD Team |

16th Annual Conference & Workshops TCS 2024

Workshops: 18th October 2024

ACTREC, Tata Memorial Centre, Navi Mumbai

Registration: 08'00~9'00

Common Lectures: Hall-KS

9'00~9'30	Immunophenotype approach to Hodgkin Lymphoma and differential diagnosis	B Wood
9'35~10'00	Quality Control in Flow Cytometry....	N Deshpande

Breakfast

Workshop-1b: Approach to AML & AML MRD - Day-2 (HALL-KS)

11'00-11'30	FCM data analysis approach to AML.	A Handoo
11'30-13'00	Live analysis of AML cases	S Johari, M Kar V Gadage
14'00-14'30	Analysis approach to AML MRD	N Patkar
14'35-15'30	AML MRD live cases	S Raj, S Thakur

Tea/Coffee Break

16'00-16'30	Rapid Fire ~ AML MRD cases	
16'35-17'00	How to diagnose ALAL: Live cases	G Chatterjee

Workshop-3b: Approach to T & NK CLPD - Day-2 (Hall-PTC)

11'00-11'30	Establishing FCM based T-clonality	A Chaturvedi
11'35-12'05	Approach to CD4+ T NHL	S Rajpal
12'10-13'00	Approach to CD8+/CD4+8+/CD4-8-T & NK-NHL	PG Subramanian
14'00-15'30	T-NHL: Histopathological Perspectives	S Epari S Rajpal, S Java

Tea/Coffee Break

16'00-17'00	T-NHL Live cases analysis	S Lakshmi, Sridevi HB P Gudapathi
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16th Annual Conference & Workshops TCS 2024

Workshops: 18th October 2024

ACTREC, Tata Memorial Centre, Navi Mumbai

Workshop~9: Approach to non-Hematolymphoid neoplasms (Hall-PS)

11'00-12'00	Immunophenotypic approach to RCT & other non-Hematolymphoid neoplasms	A Orfao
12'05-13'00	TMC experience in FCM role in RCT & other non-Hematolymphoid neoplasms.	P Tembhare
14'00-15'30	Live cases analysis – RCT & other NHL	A Orfao
Tea/Coffee Break		
16'00-17'00	Live cases analysis – RCT & other NHL – TMC	P Tembhare

Workshop~10: Cytometric Bead Array (Hall CCE)

11'00-11'20	Introduction to Cytometric Bead Array	N Deshpande
11'25-12'00	Clinical applications of Cytokine analysis	N Jindal
12'05-12'30	Standard operating procedure – CBA	K Girase
12'30-13'00	Basics of analysis and reporting results	M Bhoru
14'00-15'30	Hands-on processing and analysis in CBA	S Poojary, S Bane
Tea/Coffee Break		
16'00-17'00	Intracellular cytokine staining workflow with Dry reagent	J Yadav

16th Annual Conference & Workshops TCS 2024

Workshops: 18th October 2024

ICMR NIIH & ICMR NIRCH, Mumbai

Time 9`30~17`30

Workshop-6: continues... (NIRCH)

High Dimensional Data-Driven Discovery

- ... 30 colour panel acquisition on High Dimensional Cytometer BD Symphony A3
- ... Tutorial on FlowJo Conventional and Unsupervised high dimensional data analysis

Workshop-7: continues... (NIRCH)

Conventional & Image Enabled Sorting

- ... Hands-on & Tutorial: Conventional and Spectral Sorting

Workshop-8: Inborn Errors of Immunity (IEI) (Day-2 NIIH)

- | | |
|--|-------------|
| ... Pitfalls in Flowcytometry | K Gilmour |
| ... Functional Validation in Variant of Unknown Significance | J Aluri |
| ... Diagnostic approach to HLH | S Sabrish |
| ... Role of Flow Cytometry in Autoimmune disorders | R Abraham\ |
| ... Hands-on Perforin and Granule release assay & Analysis | S Sabrish/ |
| | S Shinde |
| ... Case-based Discussion | R Mallik |
| | A Daivi |
| | N Jodhawat |
| | D Vedpathak |
| | P Gaikwad |

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Dr Brent Wood, MD PhD
Director of Diagnostic Immunology & Flow Cytometry,
Children's Hospital Los Angeles, USA
Professor of Clinical Pathology, Keck School of Medicine
of USC, USA

Dr Brent Wood, considered one of the pioneers in the field of clinical flowcytometry and Hematopathology, was instrumental in implementing 10-color flowcytometry in clinical diagnostics and MRD monitoring. His laboratory serves as one of the nodal reference points for MRD testing in childhood leukemias for the COG group. Dr Wood has been intricately involved in developing consensus guidelines and reference books including the latest WHO5 classifications of Hematolymphoid neoplasm. Dr Wood will share his expertise in the following key areas at TCS 2024.

1. Defining lineage in acute leukemias as per WHO5 2022
2. Approaches to ALL MRD detection for patients being treated with CAR T therapy
3. Immunophenotypic approaches to MDS diagnosis
4. Immunophenotypic approach to Hodgkin lymphoma

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Dr Alberto Orfao, MD PhD

Professor, Department of Medicine, University of Salamanca
Deputy director of the Cancer Research Center (CIC/FICUS),
Director of the Cytometry and Cell-Sorting Central Services of
NUCLEUS (University of Salamanca)
Scientific Director of the Spanish National DNA Bank and the
Network of Tumor Biobanks of Castilla y Leon (Spain)

Dr Alberto Orfao is one of the most prominent contemporary immunologists and clinical flowcytometrists. Dr Orfao has made significant contributions to the rapidly evolving fields of cancer detection methods and sensitive immunophenotypic approaches to rare event analysis. His group's research activities mostly focus on precursor stages of hematolymphoid malignancies and immune dysregulation in cancer. Dr Orfao will share his expertise in the following key areas at TCS 2024.

1. Circulating tumor cells in hematolymphoid neoplasms.
2. Immunophenotyping in mast cell neoplasms.
3. Monocyte/macrophage/PDC: Implications for the diagnosis and classification of AML.
4. Maturation pattern and stem cell abnormalities in MDS & JMML.
5. New updates in immunophenotypic approaches to B-CLPDs.
6. Immunophenotypic approaches to round cell tumors and other non-hematolymphoid malignancies.

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Aaron J. Tynik, PhD

Director Scientific Affairs, Applied Research &
Technologies Team Lead at BD, USA

Dr Aaron Tynik currently works as the Director Scientific Affairs, Applied Research & Technologies Team Lead at BD, USA. His areas of expertise include high-color flowcytometry, single cell multi-omics, and developing analysis algorithms. Dr Tynik will conduct the **Spectral Flow Cytometry** workshop in collaboration with ACTREC, Tata Memoria Centre team members.

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Dr Orianne Wagner-Ballon

Professor, Department of Hematology and Immunology,
Assistance Publique-Hôpitaux de Paris,
University Hospital Henri Mondor, Créteil, France.

Dr Wagner-Ballon and team has been prominently working in the field of immunology and cancer diagnostics, and has been an integral part of several groups such as the French MDS group, and the ELN iMDS flow working group. Dr Wagner-Ballon and group were first to demonstrate the diagnostic importance of monocyte partitioning in diagnosis of CML. Dr Wagner-Ballon will discuss the **immunophenotypic approaches to CML diagnosis as well as demonstrate live CML cases** at TCS 2024.

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Dr Kimberly Gilmour

Clinical Scientist at Great Ormond Street Hospital for Children NHS Trust, London, UK

Dr Kimberly Gilmour is vastly experienced in the field of translational research in immunology and cell therapy, with a special focus on immunodeficiency. Dr Gilmour will discuss the **pitfalls of flow cytometry in detecting inborn errors of immunity** at TCS 2024.

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Dr Roshini Abraham

Professor of Clinical Pathology at The Ohio State

University College of Medicine, USA

Associate Chief of Academic Affairs and the Founding

Director of the Diagnostic Immunology Laboratory,

Ohio, USA.

Dr Roshini Abraham has worked extensively in the field of clinical immunology and has served as Past President of the Clinical Immunology Society. She is the Chair of the Document Development Committee of the Clinical Laboratory Standards Institute on Newborn Screening for Severe Combined Immunodeficiencies, Co-Chair of the Immunology Clinical Domain Working Group for ClinGen (The Clinical Genome Resource), and Co-Chair of two ClinGen Gene Curation Expert Panels. Dr Abraham's research interests focus on primary and secondary immunodeficiencies, immune dysregulatory disorders and transplant immunology. Dr Abraham will discuss the **role of flow cytometry in autoimmune disorders** at TCS 2024.

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Dr Man Updesh Singh Sachdeva
Professor, Department of Hematology,
PGIMER, Chandigarh

FCM approach to plasma cell neoplasm:

Plasma cell neoplasms (PCN) are a group of disorders resulting from proliferation of clonal plasma cells. These range from indolent lesions like monoclonal gammopathy of undetermined significance (MGUS) to pre-symptomatic smoldering multiple myeloma (SMM) to symptomatic multiple myeloma (MM) or plasmacytomas, to aggressive plasma cell leukemia. Flow cytometry immunophenotyping is an integral part of diagnosis and management of PCN. The neoplastic plasma cells have distinct immunophenotype which can be used by multicolor flow cytometry (MFC) to distinguish these from normal or reactive plasma cells (PCs).

The most widely used markers for gating of plasma cells include CD38, CD138 and CD45 in association with the forward scatter and side scatter properties. Addition of other gating markers like CD319, CD229, CD54, multi-epitope CD38 may be considered if anti-CD38 immunotherapy has been used. Other commonly used surface markers for distinguishing normal from abnormal PCs include CD19, CD27, CD56, CD81, CD117, CD200 and CD28. In addition, cytoplasmic expression of light chains provides evidence of clonality.

MFC can provide prognostic information, with presence of >95% abnormal PCs in bone marrow indicating higher chances of progression of MGUS or SMM to MM. In addition, MFC provides critical information on response to therapy by detection of minimal residual disease (MRD) with a sensitivity of 10-5 or higher. Numerous studies have shown MRD to be of indicative of shorter progression free survival (PFS) in myeloma.

MFC has proven extremely useful in assessment of burden of circulating abnormal plasma cells at the time of diagnosis and also during follow up time points, which also has been shown to be very good indicator of PFS and overall survival.

Overall, flow cytometry remains an indispensable tool for diagnosis, follow up and overall management of plasma cell neoplasms.

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Dr Khaliquir Rahman

Professor, Department of Hematology,
SGPGI, Lucknow

Flow cytometry based T-cell Clonality using TRBC1:

T-cell proliferation is one of the common manifestations in different clinical conditions which is usually associated by a skewed CD4:CD8 ratio and under-expression or overexpression of some of the T cell markers. These changes, can be associated with several reactive conditions. Flow cytometry based assessment of T cell clonality has typically been carried out in the form of VB repertoire assay, which itself is a tedious one. In last decade, there was a new attempt involving the T-cell receptor constant β chains (TRBCs) for the FCM based evaluation of T cells. A few years ago, a monoclonal antibody specific for TRBC1 was discovered. TRBC1 is one of two mutually exclusive subunits of TRBCs. In a reactive and polyclonal conditions these two subunits are proportionately represented. An overrepresentation or underrepresentation (absence) of these TRBC1 positive T cells helps in defining the T cell clonality. The addition of this antibody contributes to the detection of clonality within immunophenotypically distinct CD3+ T-cell subsets in a rapid, simple and economic way.

Testing for TRBC1 expression has traditionally been challenging because the two constant regions, TRBC1 and TRBC2, are highly similar. However, advances in flow cytometry and molecular techniques have allowed for development of antibodies specific to these two subunits. Antibodies against TRBC1 was the first to be developed. Hence, it is the one being commonly used in routine practice. In a multicolour combination, the T cells which are positive for the TRBC1 are the one which express it, and the cells which appear negative for TRBC1 are assumed to express the TRBC2. Any subset of T cell expressing more than 85% or less than 15% is most likely to be of clonal origin. It should also be noted that TRBC1/TRBC2 is a part of the TCRαβ-CD3 complex only expressed on mature αβ T-cells and γδ T-cells are inherently negative for TRBC1/TRBC2. The clonality detection using TRBC1 should always be performed in conjunction with multiple other T-cell antigens. Ideally, an 8-10 color flow panel should be used in immunophenotyping, allowing analysis of different T-cell subsets and separation of neoplastic and benign T-cells. The expression of TRBC1 should be noted on the alpha beta positive T cells only. Any expression of more than 85% or less than 15% is usually taken as the evidence of clonality. This is fortified by a loss/downregulation of other T cells related antigens, mostly CD7 & CD5.

To conclude, utility of TRBC1 in a multicolor combination is being established as one of the easy and robust marker for the clonality evaluation of the T cells.

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Dr Sweta Rajpal

Associate Professor, Hematopathology,
ACTREC, Tata Memorial Centre, Navi Mumbai

FCM markers/patterns predicting underlying genomics in Acute Leukemias:

This talk will highlight the various clues on FCM patterns/markers that help in suspecting the underlying genomics in several different acute leukemias (AL). Few important points to remember:

1. An integrated approach that evaluates different compartments such as blasts, granulocytes, monocytes, and other cells like PDC/basophils is required. For example, AMLs with *CBFB::MYH11* may show monocytes expressing aberrant CD2. Therefore, in addition to the blasts, other compartments should be evaluated.
2. It is very important to reiterate that FCM patterns are neither specific nor sensitive in majority of the situations. Therefore, although FCM helps in quick suspicion of an underlying genomic abnormality, confirmation using cytogenetic/molecular techniques is essential.

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Dr Ganesh Kumar V

Assistant Professor, Hematopathology,
AIIMS, New Delhi

Immunophenotyping in B-CLPDs:

Flow cytometry (FCM) is the most commonly used method for diagnosing mature B-lymphoid neoplasms, playing a crucial role in diagnosis, prognosis, and management decisions. FCM is typically performed in response to lymphocytosis using a lymphoid screening tube, which contains markers for B, T, and NK cells. This initial screening helps determine further testing requirements.

An optimal B-CLPD panel should include the following markers: CD45, CD19, CD20, CD10, CD5, CD79b, CD22, kappa, lambda, CD23, CD43, CD200, ROR1, CD38, CD11c, CD25, CD103, CD123, CD305, IgM, and IgD. In rare cases, markers for immaturity may be necessary to distinguish leukemic variants of DLBCL or Burkitt's lymphoma/leukemia from acute lymphoblastic leukemia (ALL).

In B-lymphoid tubes, pan-B markers such as CD19, CD20, CD10, and CD79b are employed. Key markers for initial subtyping include CD5 and CD10, while clonality assessment is conducted using surface light chains, namely kappa and lambda.

CD5-positive neoplasms include chronic lymphocytic leukemia (CLL) and mantle cell lymphoma, whereas CD10-positive neoplasms encompass follicular lymphoma and a subset of diffuse large B-cell lymphoma (DLBCL) in its leukemic phase. Neoplasms that are CD5-negative and CD10-negative include splenic marginal zone lymphoma, marginal zone lymphomas/leukemias, splenic B-neoplasms with prominent nucleoli, and hairy cell leukemia. The latter is characterized by markers such as CD11c, CD25, CD103, and CD123.

The intensity of marker expression is critical in B-mature lymphoid neoplasms; CLL, for example, often exhibits variable and heterogeneous expression of CD20 and light chains. The Matutes score is commonly used to assess CLL.

Recent advancements have introduced new markers like CD180, and CD148 to help differentiate CD5-negative, CD10-negative B-chronic lymphoproliferative disorders (B-CLPDs). Prognostic markers such as CD38 and ZAP-70 are also significant in CLL.

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Dr Karthik Bommannan B.K.

Associate Professor, Department of Oncopathology,
Cancer institute(W.I.A), Chennai

FCM markers predicting outcomes among patients with B-ALL :

According to the World Health Organization (WHO) 2022, patients with B-ALL are categorized based on their underlying recurrent genetic aberrancies (RGA) of prognostic relevance. Classification of B-ALL patients according to this prognostic-relevance model requires detailed molecular genetic evaluation, which is both time and economy consuming, and not always feasible in resource limited settings. From clinical view point, the turnaround time for results of these molecular genetic tests do not support rapid prognostication.

Flow cytometric immunophenotyping (FCI) plays a vital role in the diagnosis and follow-up of patients treated for acute leukemias. Apart from its role in lineage assessment, the expression profile of certain antigens and parameters derived by FCI assays serve as surrogates for underlying RGAs and also predict outcomes. The rapidity of FCI assays aid quick clinical decisions and also bridge the gap between the shortcoming of molecular tests (cost & TAT) and patient care.

At diagnosis, over expression of CD20, CD34, CD45, absence of CD10 expression (common with KMT2A rearranged B-ALL), expression of myeloid antigens (surrogate for B-ALL with BCR::ABL1) and CRLF2 (surrogate for B-ALL with BCR::ABL1 –like features) are associated with poor outcomes. However, expression of CD371 (surrogate for DUX4 rearrangement), absence of CD45, over expression of CD123 (both associated with high-hyperdiploidy) are associated with better prognosis. Parameters like DNA-index and blast size index are very helpful in ploidy-based risk assessment in patients with B-ALL. There are Indian treatment protocols which have used only DNA-index as a tool for ploidy-based risk assessment, instead of cytogenetics and FISH.

During follow up, the independent prognostic relevance of measurable residual disease determined by FCI is well established. In a relapse-refractory setting, FCI derived information regarding the expression of CD22, CD19 and CD20 antigens facilitate the use of monoclonal anti-body and CAR-T cell therapies that are detrimental in improving patient outcomes.

16th Annual Conference & Workshops TCS 2024



Dr Nabhajit Mallik

Assistant Professor, Department of Hematology,
PGIMER, Chandigarh

FCM-based baseline risk-stratification in ALL:

It is well known that minimal/measurable residual disease analysis, commonly performed by flow cytometry, is the strongest prognostic indicator in acute lymphoblastic leukemia (ALL). However, the parameters routinely used for baseline risk-stratification in ALL include age (especially in children), total leucocyte count, extramedullary site involvement (CNS/testicular) and cytogenetic/molecular abnormalities. In this background, does flow cytometry have any role to play in ALL risk-stratification at baseline?

Well, if you look closely, the recurrent genetic abnormalities in B-ALL include high-hyperdiploidy, which have a favourable prognosis, and hypodiploidy, having poor prognosis. Traditionally, ploidy analysis is performed using conventional cytogenetics. However, flow cytometry-based DNA ploidy analysis is an excellent and rapid method for determining DNA ploidy by calculating the DNA index. DNA-binding dyes do have their technical limitations, however newer dyes such as FxCycle violet give excellent results even with 6-8 colour panels. A pitfall of ploidy analysis is the doubling of near-haploid or low-hypodiploid clones, resulting in pseudohyperdiploidy, and cases having two peaks need to be carefully evaluated.

In addition to the above, certain flow cytometry markers point towards a particular genetic subtype, and thus may provide indirect prognostic information. A good example is CD10 negativity (along with CD15 and NG-2 expression), which correlates well with KMT2A rearranged B-ALLs, and thus indicates a poor prognosis. For T-ALLs, a specific subtype having poor prognosis is the early T-cell precursor lymphoblastic leukemia (ETP-ALL), which although initially described by gene expression profiling, is now defined by immunophenotypic characteristics, including absence of CD8 and CD1a, absent/dim CD5 expression, and presence of one or more myeloid and/or stem cell markers. Thus, we can conclude that although the role of flow cytometry in risk-stratifying ALLs at baseline is limited compared to molecular genetics/cytogenetics, it does have great utility in certain situations.

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Dr Devasis Panda

Consultant Hematopathologist,
RGCIRC, New Delhi

FCM approach to B-ALL MRD:

Minimal residual disease has emerged as a robust prognostic indicator in both adult and pediatric B-cell ALLs. Flow cytometry plays a pivotal role in MRD detection in B-ALL and has evolved from simple panels consisting of few basic B-cell maturation markers into bigger exhaustive panels with an ever-increasing number of LAIP markers. The approach to MRD detection in B-ALL is principally based on deviation from normal expression patterns at each stage of B-cell ontogeny as well as aberrant expression of lineage infidel markers. A good understanding of both of these aspects is essential for efficient MRD detection in B-ALL.

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Dr Avinash Gupta
Consultant Hematopathologist & Co-Director,
Gwalior Hematology Centre, MP

FCM approach to T-ALL MRD:

Introduction: MRD testing aims at detecting and quantifying residual blasts beyond the sensitivity of cytomorphology. MRD positivity is the single most powerful high-risk factor in acute leukemia including T-cell acute lymphoblastic leukemia.

Why T-ALL MRD: MRD-positive status in T-ALL is significantly associated with inferior relapse-free survival (RFS) and overall survival (OS).

Methods for MRD detection: Commonly used techniques include Multicolor flow cytometry-based MRD (MFC-MRD) to detect leukemic cells by immunophenotypic aberrancies, real-time quantitative polymerase chain reaction (qPCR) for detection of recurrent gene fusions or rearranged immunoglobulin (IG) and T-cell receptor (TCR) genes.

Advantages of MFC MRD: MFC-MRD is a rapid, simple & highly sensitive technique for MRD assessment and has peculiar advantages like familiarity of technique (as it is routinely used for the diagnostic work-up), wider availability, relatively inexpensive method and higher applicability compared to PCR-MRD. Though PCR-MRD has been claimed to be more sensitive, however it is known for challenges like limited availability, time consuming, labour-intensive methodology, need for diagnostic samples and limited applicability.

Approach to MFC MRD: Theoretically, T-ALL MRD seems to be very easy, since any immature T cell detected in BM or PB will be considered as MRD. However, sometimes detection of T-ALL MRD can be very challenging due to absence or very dim expression of T-cell immaturity markers (CD1a, CD34, CD99, TdT) and brighter CD45 expression in the residual leukemia after treatment. MFC-MRD using approach of exclusion is easily adaptable and more useful in eliminating various mimickers and artifacts.

Conclusion: T-ALL MRD has a clinical implication in management of T-ALL. Proper template designing and choosing appropriate gating strategy will ease the MRD detection. Before approaching MFC based T-ALL MRD, knowledge of various CD7 positive subsets and major confounders like immature NK cells (iCD3+ and CD16- CD56-) is a must.

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Dr Anu Singh
Senior Resident, Department of Hematology,
SGPGI, Lucknow

FCM approach to bone marrow involvement by B-NHL:

The accurate diagnosis of B-NHLs requires a multifaceted, multidisciplinary approach. A crucial component of this process involves assessing BM involvement. Traditional methods for evaluating BM, such as PET-CT and trephine biopsy histopathology, often exhibit limitations in sensitivity, leading to a high rate of false negatives.

Multicolour flowcytometry (MFC) for BM involvement by B-NHLs offers a valuable tool for diagnosis, subtyping, staging, prognostication, and the detection of occult disease. While literature is limited, even minimal bone marrow involvement has been correlated with an elevated International Prognostic Index (IPI) and unfavorable clinical outcomes. MFC enables a comprehensive immunophenotypic evaluation, simultaneously assessing both surface and cytoplasmic markers. A skewed light chain ratio, aberrant antigen expression, or the absence of both light chains in a homogenous population of mature B cells should raise suspicion and warrant confirmation with additional markers. Lymphomas exhibiting the distinctive immunophenotype of CD5+/CD10+ can still be reliably identified through the application of an appropriately tailored panel of markers. However, the detection of low-level bone marrow involvement in cases lacking characteristic immunophenotypic deviations, can present a diagnostic challenge. A crucial factor in detecting minimal involvement is the acquisition of a substantial number of events; a minimum of 1.5 million, ideally reaching 5.0 million, is recommended. A broad and flexible gating strategy should be employed to accurately identify normal cell populations, precursors, intermediate stages, and potential abnormal clusters. The proposed panel includes CD45, CD19, CD20, CD5, CD10, CD38, CD305, CD34, CD3, CD4, CD7, CD8, TCR-GD, CD14, CD16, CD56, and light chains kappa and lambda. CD305 is a pivotal antigen marker, that functions as negative regulator of B cell receptor signalling. Its downregulation is instrumental in identifying occult bone marrow involvement, particularly in lymphomas lacking a distinct immunophenotype, such as CD5- and CD10-negative lymphomas.

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Dr Manisha Madkaikar

Director and Scientist G

ICMR-National Institute of Immunohematology and

ICMR-Centre for Research

Area of expertise- Inborn Errors of Immunity and Haemoglobinopathies

Title: Primary immunodeficiencies: Overview and current trends.

Dr Manisha Madkaikar leveraged 2 decades of experience working in this area to inform a diverse audience on various aspects related to using flow cytometry in the diagnosis of various primary immunodeficiencies. This included design of high parameter immunophenotyping panels addressing multiple cellular immune subsets such as memory T and B cells as well as activated NK cells. Further gating strategies and quality control measures adopted during data analysis and reporting were also discussed.

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Dr. Urmi Chatterjee
Professor, Department of Zoology
University of Calcutta

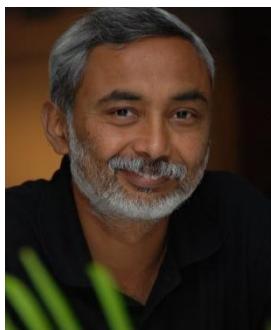
Area of expertise- Cancer Biology

Title of the Talk- Stem cells and Cancer: what's new!

Dr Chatterjee spoke about the state of four basic areas, using flow cytometry, in the area of cancer stem cell biology which were:

1. Telomerase inhibition as potential anti-cancer therapeutic strategy
2. Reversing arsenic-Induced endocrine disruption with retinoic acid: A therapeutic strategy
Investigating the molecular dynamics of progression of prostate cancer
3. Effect of arecoline on the gene expression of androgen receptor in the prostate of male rats
4. Delivery of antisense oligonucleotides to the androgen receptor of prostate cancer cells by nanoparticles a prospective anti-tumoral strategy

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H. Krishnamurthy

Advisor, Central Imaging & Flow Cytometry Facility
National Centre for Biological Sciences, Tata Institute of
Fundamental Research

Area of expertise- Reproductive Biology

Dr. Krishnamurthy is a founding member, former General Secretary (Research) as well as recipient of the TCS Oration award of The Cytometry Society of India. At present, he is working as Scientific Officer "F" and in charge of Flow Cytometry and Confocal Microscopy Facilities at the National Centre for Biological Sciences, Tata Institute of Fundamental Research, Bangalore, India. His research interest lies in hormonal regulation of spermatogenesis and trafficking and signaling of gonadotropin receptors. Our esteemed keynote speaker 'Krishna' presented his talk on '**Basics of Flow cytometry: from photons to cells**'. He introduced the participants to varied aspects and principles governing the sophisticated technology of Flow cytometry.

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Anugya Bhatt

Scientist G, Sree Chitra Tirunal Institute
for Medical Sciences 7 Technology

Area of expertise- 3 D Bioprinting, Stem Cell biology, Cellular secretome, Cell-Cell interaction

Title: Cell free therapeutic approach for impaired wound healing.

Dr. Anugya Bhatt currently works at the Thrombosis Research Unit, Sree Chitra Tirunal Institute for Medical Sciences and Technology. Her research spans Biochemistry, Biology and Biophysics. She is currently working on Skin Tissue Engineering, Blood compatibility and Platelet Biology. Her talk dealt with 3D Bioprinting of skin tissue constructs: Dual layer skin tissue construct were printed and analyzed in-vitro and in-vivo. In-vitro studies showed functional skin markers and the preliminary in-vivo studies demonstrated better wound healing. She also shared some data with respect to mesenchymal stem cells and activated platelets and the role of secretome in wound healing. Finally, she informed the audience about some exciting results relating her work on the development of the PT INR assay platform: A novel device developed to measure prothrombin (clotting) time.

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G SRINIVAS
Senior Technical Officer
Centre for Cellular and Molecular Biology

Area of expertise- FACS sorting

Srinivas ji has been working in the Central FACS facility of CCMB for the past 24 years. He has vast experience in cell sorting and analysis of biological samples through high-speed sterile sorters for further cultures or to isolate nucleic acids/proteins and other organelles. He provides training to in-house students' / project staff as well as support in designing and analysis of experiments to all investigators / local scientific community. His contribution in various research groups such as Cell biology, Stem cell biology and Infection biology has helped the users to publish their work in various high impact journals. Besides teaching & supporting, he contributed to the development and optimization of various aspects of the FACS SOP's, experiment- related protocols methodology. He also has expertise of handle various flow cytometry softwares like Cell Quest Pro, FACS Diva , Summit, Kaluza, FACS express, WinMDI,

TITLE AND ABSTRACT OF THE TALK

Basics of cell sorting:

Cell sorting using FACS is an art and requires wide knowledge starting from sample preparation, machine calibration for sorting setup, and the terminology used like **laser delay, drop delay, amplitude and frequency** while sorting of the cells. Learning about various mode of sorting will help in designing and planning the experiment for sorting to get maximum number without losing population of interested cells. It is also important to know the setting of threshold and **double cell discrimination, gating strategies while using different sorting modes like purity, enrich and single cell sorting with maximum efficiency**. Various factors of sample related and machine related issues will effect the sorting efficiency will be discussed.

This workshop will be focused to get understand above mentioned terminology used in Cell sorting by FACS techniques.

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Dr. Tarannum Mubin

Scientist

Dana-Farber Cancer Institute,
Harvard Medical School

Area of expertise- Nanoparticle therapy, Oncology

Title of the talk- Cellular Therapy in ovarian cancer

Dr Mubin was a special 'Early stage investigator' invitee to the meeting based on her exciting work in the area of solid tumor directed CAR-T development conducted at the Dana-Farber Cancer Institute, Boston, MA. She shared her expertise in the design of experiments for making various modifications of nanoparticles. Furthermore, she has demonstrated the ability in articulating tailored nanotechnology-based therapeutics and nanomedicine to meet the demands in oncology. Highly skilled in conducting in vitro and in vivo studies for cancer therapeutics. In this talk she highlighted a novel memory like NK cell CAR developed for the treatment of ovarian cancer.

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Dr Rajesh Pandey

Principal Scientist

CSIR-Institute of Genomics and Integrative Biology

Area of expertise- INtegrative GENomics of HOst-PathogEn (INGEN-HOPE), Functional genomics, Non-coding RNA Biology, Single Cell Genomics, Transcriptionally Active Microbes (TAMs), Innovative, cost-effective application of NGS technologies

Title: Internal and external cellular dynamics reveal insights into differential infectious disease severity

Dr. Rajesh Pandey is currently working as Principal Scientist at CSIR-Institute of Genomics and Integrative Biology (CSIR-IGIB) and Associate Professor, at Academy of Scientific & Innovative Research (AcSIR), Delhi, India. His area of expertise and research interest focuses on INtegrative GENomics of HOst-PathogEn (INGEN-HOPE), Functional genomics, Non-coding RNA Biology, Single Cell Genomics, Transcriptionally Active Microbes (TAMs), Innovative, cost-effective application of NGS technologies. In his keynote talk he presented distinct, omics derived patterns of host-pathogen interactions governing pathologies of COVID-19, Dengue and intracellular fungal infections.

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Dr Paresh Jain
Associate Director,
Clinical Solutions Platform,
Greater (Asia)
Becton Dickinson & Co

Area of expertise- Flow cytometry, hemat-oncopathologist

Title: Intertwined paths of standardization, automation and AI in flow cytometry

Dr Paresh Jain is a hemat-oncopathologist currently working in Singapore as Associate Director, Medical Affairs, BD Biosciences Central and South Asia and Japan. He is an active member of the ISAC's Live Education Task Force and a founder member of The Cytometry Society, India. In his talk, Dr Jain highlighted his role in institutionalizing CD4 GLP training programs for NACO sites as well as more current efforts to increase automation and AI guided procedures to improve flow cytometry based diagnosis.

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Dr Nimesh Gupta
Senior Scientist
Chief, Vaccine Immunology Laboratory
BRIC-National Institute of Immunology
Department of Biotechnology
Ministry of Science and Technology, Govt. of India

Area of expertise- Immunology of human viruses and vaccines, development of T-cell assays for testing vaccines and bio-therapeutics, and conducting clinical trials for vaccines.

Title: Navigating T cell landscapes with flow cytometry

Dr. Gupta is a virologist and immunologist with over two decades of expertise in mosquito-borne virus infections, such as Dengue and Japanese encephalitis. He leads a research group at NII, focusing on understanding the T-cell determinants of long-lasting, protective humoral immunity, with the goal of leveraging this knowledge for the rational design of vaccines. His work has resulted in over 50 publications and several patents related to T-cell-based bioassays and their application in vaccine development. In his keynote talk he highlighted some novel flow based assays that were being used to tease out protective correlates of dengue infection and rationally guide vaccine design.

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Dr Naresh Sachdeva

Professor

Dept. of Immunopathology, Post Graduate Institute of Medical Education and Research, Chandigarh, India

Area of expertise- Cell Culture, Flow Cytometry, Tissue Culture, Protein Expression, and Assay Development

Title: Engineered exosomes from Tregs: novel therapy for type 1 Diabetes (T1D)

Professor Naresh Sachdeva received his basic training in Immunology and Molecular biology from Post Graduate Institute of Medical Education and Research (PGIMER), Chandigarh, India and University of Miami, FL, USA. He is in-charge of the Molecular Immunology and Endocrinology Laboratory at PGIMER. He has research interests in pathogenesis and exploration of immunotherapeutic approaches in autoimmune diabetes. In his key note presentation Professor Sachdeva shared some recent data from his lab regarding the development of checkpoint marker loaded exosomes integrated into autologous CD4+ Tregs for T1D immunotherapy.

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Dr Albeena Nisar
ACTREC, TMC

Area of expertise- Molecular Immunology

Title: Making CAR T cells in India for India.

As part of the pioneering effort towards indigenous development of cellular therapy for Cancer in India, Dr Nisar is a rising star in the field of CART development. She explained the entire process, ground up, of how a CAR-T lab was built. This included making ethics committee submissions to designing and working with vendors to construct the facility as well as the conduct of pre-clinical studies and navigating the intricacies of securing regulatory approvals for clinical trials, and the tireless pursuit of funding through grant submissions. She covered all the aspects of CART Cell therapy from bench-to-bedside, garnering participant's attention towards the recent developments and GMP in CAR T cell research.

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Dhiraj Kumar, PhD
Group Leader, Cellular Immunology, ICGEB

Area of expertise- Immunology and microbiology

Title: Innate immunity in tuberculosis pathogenesis.

Dr Kumar focussed his key note talk on using high throughput approaches and cutting edge experimentation coupled with integrative analytical strategies to understand the interplay of host mediated inflammation and modulation thereof by M. tb with special reference to innate immunity.

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Aaron J. Tynik, PhD

Director Scientific Affairs, Applied Research &
Technologies Team Lead at BD, USA

Title: BD FACSDiscover S8 imaging and spectral workflows: enabling novel applications

Dr Aaron Tynik currently works as the Director Scientific Affairs, Applied Research & Technologies Team Lead at BD, USA. His areas of expertise include high-color flowcytometry, single cell multi-omics, and developing analysis algorithms. Dr Tynik conducted the **Image Enabled Sorting** workshop in collaboration with NIRCH. He primed the participants about BD FACSDiscover, a new platform to explore new depths of spectral cell sorting with imaging and new possibilities in data analysis.

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Oral abstracts (Clinical):

CO-01: Evaluating the Impact of Flow Cytometry on Platelet Function Disorder Diagnosis: Insights from a 5-Year Retrospective Study in a Tertiary Care Center.

Anusha Thangraja, Nitty S Mathews, Sukesh C Nair, Ramya V

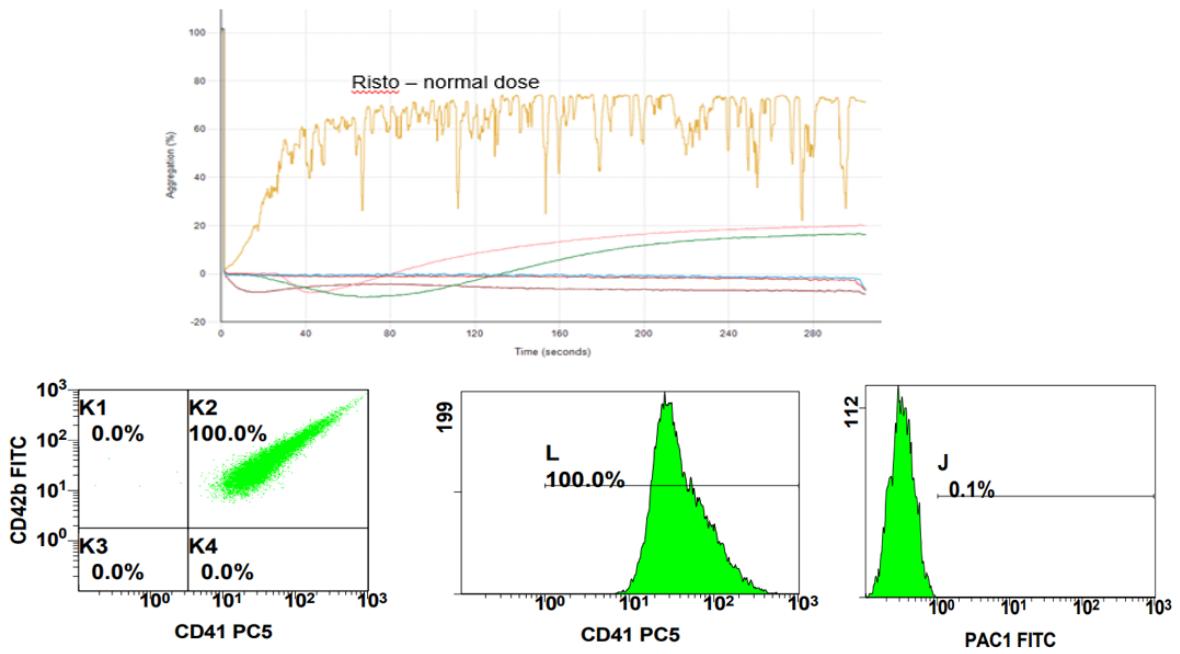
CMC Vellore

BACKGROUND: Platelet function defects (PFDs) are heterogeneous in pathogenesis and pose diagnostic challenges. In this study, we aim to evaluate the benefits of incorporating flow cytometric platelet function testing alongside standard LTA screening in patients suspected of having platelet function disorders (PFD).

MATERIALS AND METHODS: A 5-year retrospective observational study was conducted on patients suspected of platelet function disorders from January 2019 to July 2024. Clinical information, ISTH BAT score, clot retraction test, PFA 200, aggregometry and flow cytometry-based platelet function test findings were analyzed for all patients. Flow cytometry-based platelet function testing was performed using Beckman Coulter – Navios (8-color) flow cytometer. Antibodies used were CD 41, CD42b, Anti VWF, PAC1, CD 62P, CD 63, Agonist -ADP, Convulxin, Ristocetin, TRAP and mepacrine.

RESULTS: Out of 3,745 patients evaluated for coagulation disorders, 387 (10%) were suspected of having platelet function disorders. 47% (183) cases of Glanzmann thrombasthenia (GT), 12% (48) of Bernard Soulier Syndrome (BSS), 21% (80) of platelet secretion/granule release defect, 14% (53) of acquired platelet dysfunction with eosinophilia (APDE) and 6% of others. The use of flow cytometry-based platelet function tests allowed us to classify 11 cases (6%) of GT into GT Variant (normal CD41 and absent PAC1) and Type 2 GT (dim CD41 and PAC1 expression). Additionally, we identified cases of co-existing APDE alongside a case of BSS and Quebec platelet disorder, enhancing our diagnostic findings. The evaluation of CD62p, CD63, and the mepacrine assay following agonist stimulation enabled us to confirm the diagnoses of Grey platelet syndrome, delta storage pool disease, mixed granule deficiency, ADP P2Y12 receptor defects, thromboxane receptor defects, mild platelet signal transduction defects and APDE. Additionally, the use of Annexin V facilitated the identification of procoagulant platelets and aided in the diagnosis of Scott syndrome. Among the 46 cases of thrombocytopenia with suspected PFD, 13 (28%) were identified with platelet function disorders, with flow cytometry being the sole testing method used for these cases.

CONCLUSION: Our study confirmed the effectiveness of flow cytometric platelet function testing in diagnosing platelet function disorders and thrombocytopenia and can be regarded as a valuable tool in the diagnostic evaluation of PFDs.



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Oral abstracts (Clinical):

CO-02: Flow cytometry based MRD assessment in Acute Myeloid Leukemia: A powerful tool to predict treatment outcome.

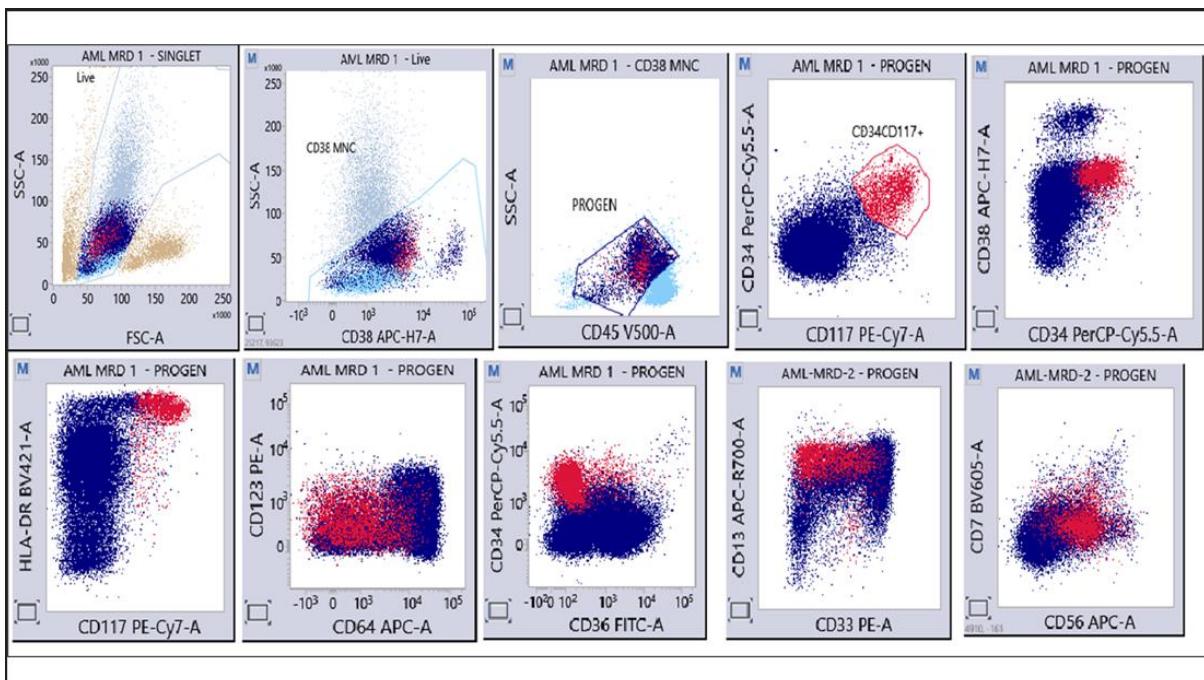
Kusum Gupta, Ruchi Gupta, Khalique rahman, Manoj Sarkar, Manish Kumar Singh, Dinesh Chandra, Gala deep, Sanjeev, Rajesh Kashyap
SGPGIMS, Lucknow

Introduction: MRD in AML is considered as an independent predictor of relapse and poorer survival outcomes. FCM is a powerful and cost-effective tool for MRD assessment with high sensitivity and rapid turnaround time.

Material & Methods: This was an observational study which analysed the FCM based MRD evaluation in AML patients and its correlation with the overall survival. Different time points for MRD analysis were post Induction (PI), post consolidation (PC), pre transplant (PT) as well as DAY-100 post-transplant (AT). First pull marrow was processed using bulk lyse-stain-wash protocol and stained with two tubes 10 colour antibody panels comprising of CD45/CD34/CD38/HLADR/CD117/CD13/CD33/CD36/CD56/CD64/CD7/CD123/CD15/CD2/CD19. A target of 1-2 million cells per tube were set for the acquisition. An MRD value of 0.1% of the live gated cells were considered as the POSITIVE according to the clinically relevant cutoff.

Results: A total 206 MRD analysis were done in 125 patients, out of which in 35 patients MRD were done in multiple times (2-4 times). 45 samples were MRD positive in 30 patients (33.80%). Different from normal approach was helpful in 15 samples, while LAIP was helpful in 30 samples. Most common aberrancy were expression of CD123(59.13%), CD56 (46.15%) followed by CD7(11.53%) in LAIP approach while in dfN approach most common aberrancy was that of CD64(45%), CD36(9.5%) & loss of CD13(10%), dim CD45 (12.9%). In 05 cases, pretransplant MRD were not detectable, while day 100 post-transplant MRD was positive. Three out of them later developed frank marrow relapse. All these relapsed cases were associated with poor genetic profile. Post-induction MRD was available for 39(53.4%) out of 73 patients. 20(51.28%) patients were MRD Negative. Post-induction OS was significantly better for patients with MRD negative remission compared to MRD positive (Median OS not reached vs 18 months [95% CI 2.9-33], P=0.038). OS at 24 months for MRD negative and MRD positive patients was 90% and 43% respectively.

Conclusion: This sensitive assay of MFC based MRD analysis identified ~ 48.71% MRD positivity in the PI samples. Most frequent aberrant phenotype was CD123/CD56/CD64/CD7/CD36. Patient having MRD positivity had a significantly poor overall survival as compared to MRD negative cases.



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Oral abstracts (Clinical):

CO-03: Developing a new Objective criterion for Lineage assignment in Acute Leukemia by Flow cytometry.

Sneha Ann Oommen, Simpy Raj, Aastha Kalra, Shubham Pawar, Steffi Varghese, Sitaram Ghogale, Nilesh Deshpande, Karishma Girase, Jagruti Patil, Sweta Rajpal, Gaurav Chatterjee, Nikhil Patkar, PG Subramanian, Sumeet Gujral, Prashant Tembhare
ACTREC, Tata Memorial Centre

Introduction: Gold standard for lineage assignment (LA) in acute leukemia is flow cytometric immunophenotyping (FCI). According to the 5th Edition-WHO, LA is based on the intensity of expression (IOE) of lineage-specific marker in blasts exceeding 50% of respective mature cells. However, it is limited to relative inter-observer subjectivity. Hence, we developed a new objective and accurate criteria for LA in acute leukemia.

Methods: The IOE of lineage-specific markers including CyCD3, CyCD79a, surface-CD22 and cytoplasmic-Anti-MPO were evaluated in 1272 acute leukemia (444 B-ALL, 311 T-ALL, 469 AML and 48 MPAL) using 13-color FCI on DxFLEX flow-cytometer and analyzed on Kaluza-softwareV2.1. Median fluorescence intensity (MFI) of markers in blasts was taken at level of expression in brightest part of gated blasts that included minimum 50 events (shown in Figure-1). A normalized MFI (nMFI) for these markers was derived as a ratio of blasts-MFI/ internal negative-control MFI). A criterion of LA for each marker was selected as highest nMFI in blasts of acute leukemia of other lineage, for example nMFI for cyCD3 was selected based on highest nMFI of BALL and AML blasts. Sensitivity and specificity of these criteria was calculated.

Results: The criteria for CyCD3, CyCD79a, surface-CD22 and cytoplasmic-Anti-MPO were 16, 45.31, 23.50 and 5.53 and their highest nMFI in T-ALL was 634.03, in B-ALL was 306.58, 734.42, and in AML was 272.51 respectively. The criteria were applicable for CytoCD3 in all cases of T-ALL, CyCD79a, surface-CD22 in all cases of B-ALL, cytoplasmic-Anti-MPO in 61.08% cases of AML (rest being AML-M0, M5, M6). It was also applicable to all MPAL cases. The sensitivity and specificity were 98.29% and 99.75% for B lineage markers, 97.80% and 98.06% for CyCD3 and 98.82% and 98.56% for Cytoplasmic Anti-MPO.

Conclusions: We developed new objective criterion for LA in Acute Leukemia. These criteria values are notably lower than the 50% intensity threshold proposed by 5th Edition-WHO with very high sensitivity and specificity, suggesting a need for refinement in current lineage assignment criteria. These findings need further validation in diverse settings to confirm its applicability and reproducibility.

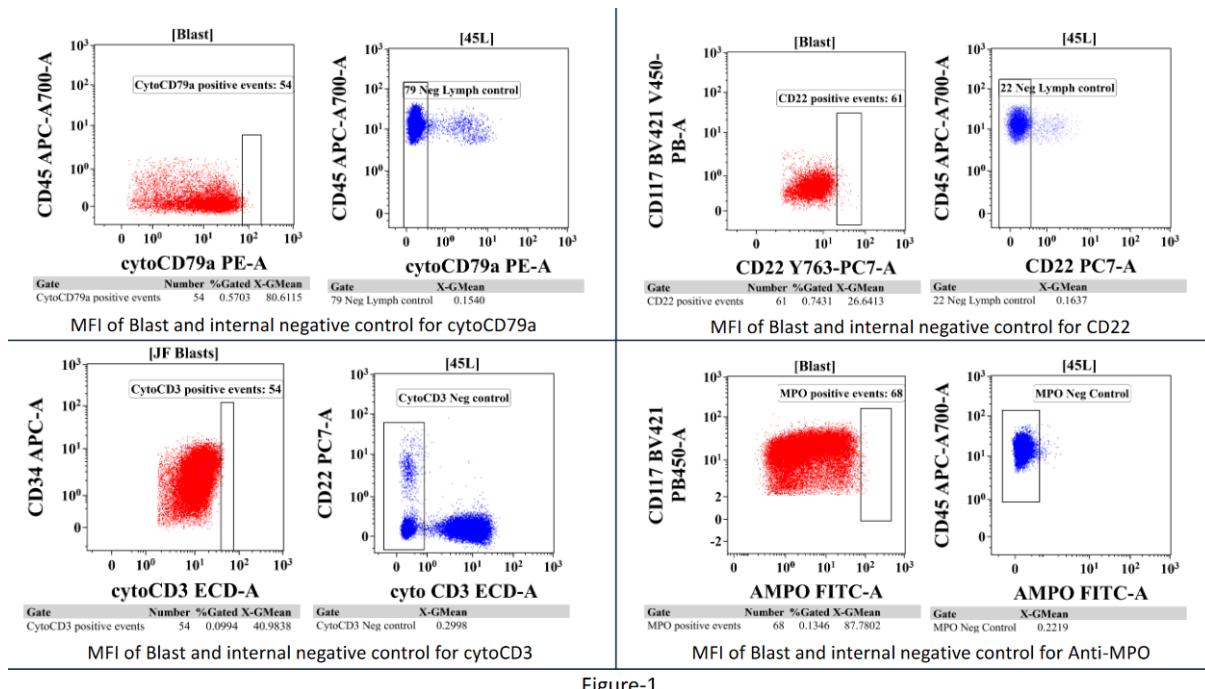


Figure-1

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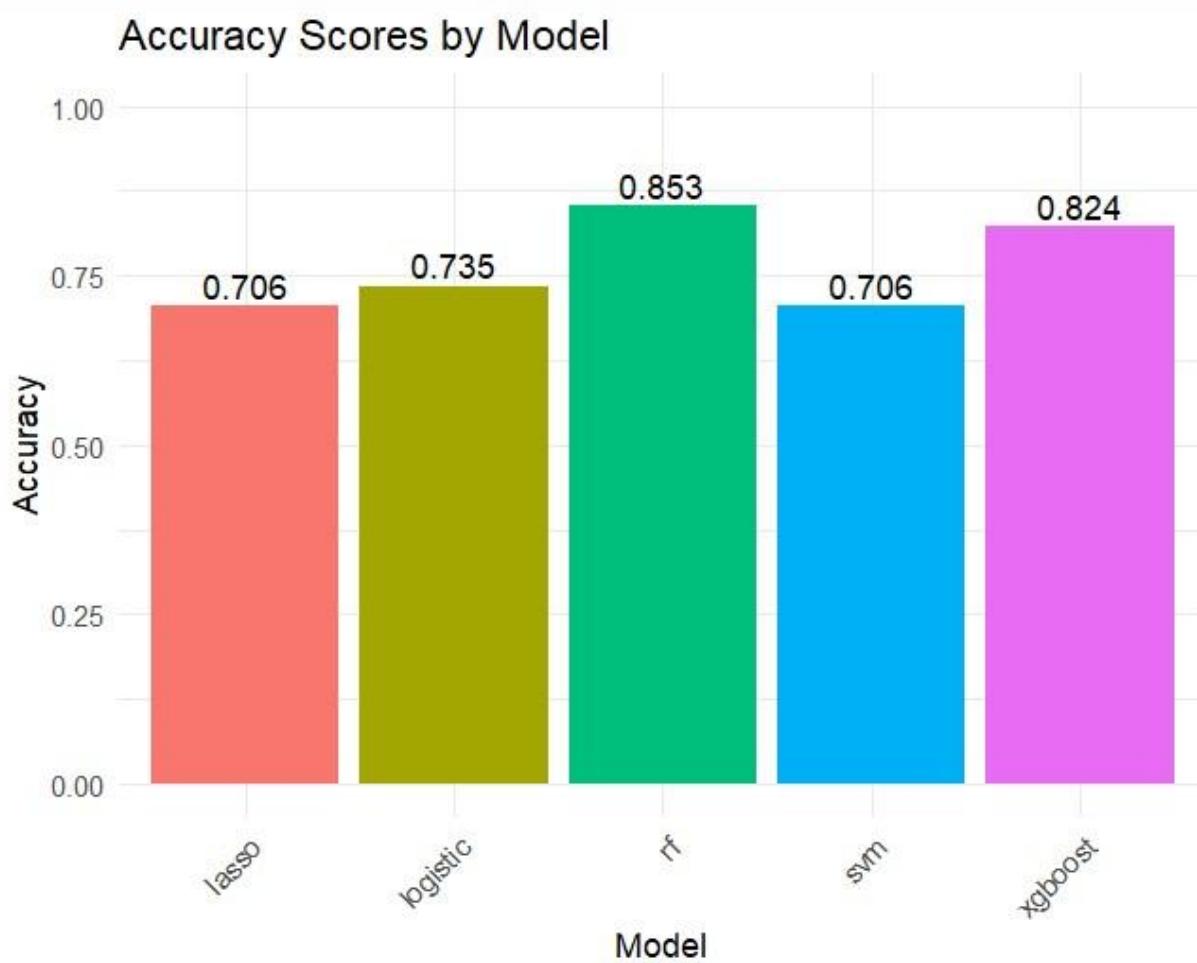
Oral abstracts (Clinical):

CO-04: Common Variable Immunodeficiency Disorder: A Decade of Insights from a Cohort of 150 Patients in India and the Use of Machine Learning Algorithms to Predict Severity.

Umair Ahmed Bargir, Priyanka Setia, Mukesh Desai, Chandrakala S, Aparna Dalvi, Shweta Shinde, Maya Gupta, Neha Jodhawat, Amruta Jose, Mayuri Goriwale, Reetika Mallik Yadav, Disha Vedpathak, Lavina Temkar, Shehal Shabirish, Gouri Hule, Vijaya Gowri, Prasad Taur, Amita Athawale, Farah Jijina, Shobhana Bhatia, Manas Kalra, Meena Sivasankaran, Sarath Balaji, Punit Jain, Sujata Sharma, Harikrishnan G, Gaurav Narula, Ratna Sharma, Pranoti Kini, Mamta Mangalani, Abhishek Zanwar, Himanshi Choudhary, Narendra Kumar, Ujjawal Khurana, Ashish Bavdekar, Girish Subramaniam, Revathi Raj, Subhprakash Saniyal, Abhijeet Ganpule, Nitin Shah, Tehsin Petiwala, Prawin Kumar, Venkatesh Pai, Sagar Bhattad, Abhinav Sengupta, Manish Soneja, Dnyaneshwar Upase, Indrani Talukdar, Manisha Madkaikar*

ICMR National Institute of Immunohaematology, Mumbai

Common Variable Immunodeficiency (CVID) is a heterogeneous disorder characterized by impaired antibody production and recurrent infections. This study investigated the clinical and immunological features of CVID in 150 Indian patients over a decade at a tertiary care center. The median age of diagnosis was 18 years, with a male predominance (62%). Most patients (66.6%) presented with a severe phenotype, with recurrent respiratory tract infections being the most common clinical manifestation (84.2%). Gastrointestinal complications and autoimmune manifestations were observed in 45% and 21% of patients, respectively. All patients exhibited hypogammaglobulinemia, with varying IgA and IgM levels. B-cell analysis revealed reduced class-switched memory B cells in 64.4% of patients. Nine adult patients presented with late-onset combined immunodeficiency. Genetic testing, performed on 52 patients, identified underlying monogenic causes in 29 pediatric and 15 adult patients, with LRBA deficiency being the most common genetic defect. We also developed a novel machine learning-based severity prediction model for CVID patients, utilizing lymphocyte subsets, class-switched memory B cell counts, and serum immunoglobulin levels. Random Forest outperformed other models, achieving an accuracy of 0.853 (95% CI: 0.840-0.866). Feature importance analysis identified Th-Tc ratio, CD19, and IgM levels as the most influential predictors for severity prediction. This study highlights the diverse clinical and immunological features of CVID in Indian patients, emphasizing the need for early diagnosis and individualized management strategies. The severity prediction model based on commonly available immune parameters may help in directing treatment strategies to improve patient outcomes. These findings contribute to a better understanding of CVID in the Indian population and offer a potential tool for clinical decision-making.



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Oral abstracts (Clinical):

CO-05: Intraoperative Flow cytometry in detecting free carcinoma cells in peritoneal washings of gastric carcinoma cases.

Thulasi Raman Ramalingam, Bharanidharan, Lakshman V, Ajit Pai

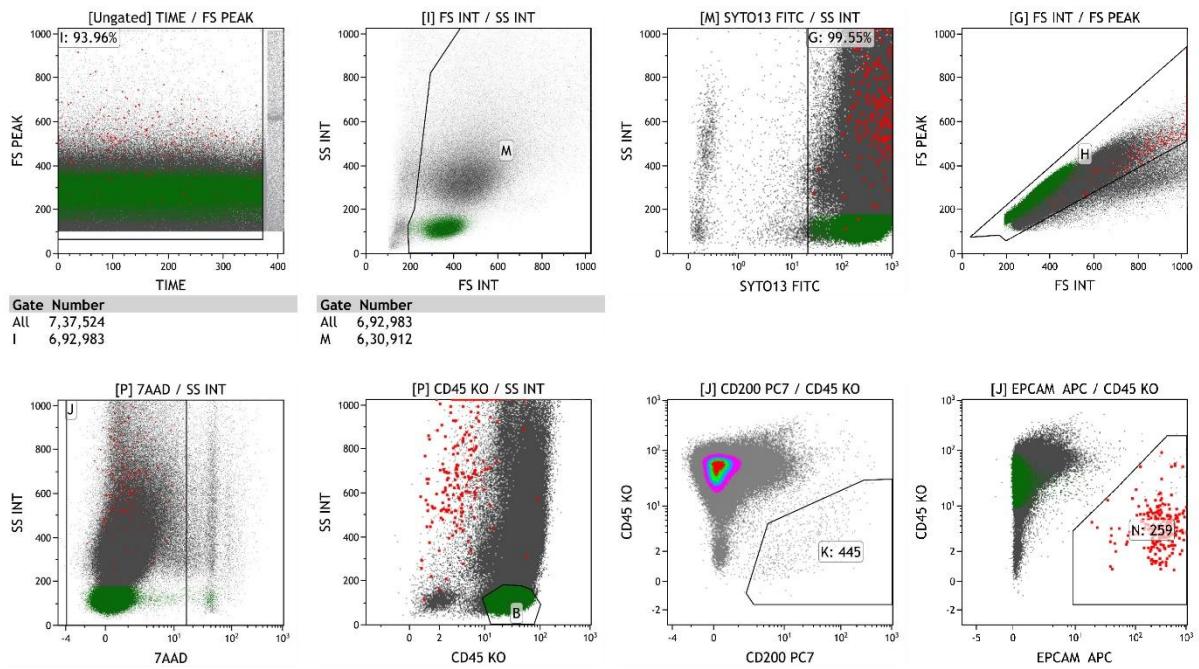
Apollo Cancer Institutes, Chennai

Introduction: Flow Cytometry (FCM) is not routinely used in non-hematopoietic neoplasms. Detection of free carcinoma cells in peritoneal washings in patients with abdominal (gastric) carcinomas is critical to decide on curative cytoreductive surgery, and currently it is done by intra-operative cytology. The free tumor cells in peritoneal washings is an independent adverse prognostic factor and indicator of postoperative intra-peritoneal recurrence in patients with gastric carcinoma. Though cytology is routinely used to assess free tumor cells, its sensitivity is poor, hence there is a need for better sensitive techniques. We attempted to study the efficiency of FCM in detecting free carcinoma cells in peritoneal washings in cases of gastric carcinoma.

Methods: In this prospective study, 30 peritoneal lavage samples of gastric carcinoma patients were analysed by cytology/Cell block and high acquisition FCM after subjecting to inclusion and exclusion criteria. A standard procedure for peritoneal lavage at diagnostic laparoscopy was followed for all patients. We developed a simple and robust high sensitive single tube, exclusive surface stain FCM (lyse-stain wash) protocol for detecting carcinoma cells. This protocol was already validated in effusion samples.¹ The sensitivity of the assay was established using LOB, LOD and LLOQ.

Results: The median time taken for processing the samples was 47 minutes. The average number of events acquired were 614781. The concordance was achieved in 84% (25/30) of samples. FCM detected carcinoma cells in Thirteen peritoneal washings of which only ten were reported positive by cytology/cell block. Two cases which were felt suspicious by cytology was negative by FCM. Omental biopsy was negative for malignant cells in these two cases. The least positivity detected by FCM was 0.005% which was negative by cytology.

Conclusion: Intra-operative flow cytometry improves the detection of carcinoma cells in peritoneal washings, thereby deciding on judicious cytoreductive surgery. The sensitivity and rapidity of FCM promises to be a good adjuvant to cytology in intra-operative diagnosis



16th Annual Conference & Workshops TCS 2024

Oral abstracts (Basic):

BO-01: Modulation of HIV-1 pathogenesis by latent TB infection: Immunological correlates.

Shilpa D. Bhowmick, Pratik Devadiga, Sapna Yadav, Pranay Gurav, Nandan Mohite, Varsha Padwal, Namrata Neman, Satyajit Musale, Snehal Kaginkar, Amit Kumar Singh, Shantanu Birje, Shilpa Kerkar, Leena Tendulkar, Vidya Nagar, Priya Patil, Sachee Agrawal, Sushma Gaikwad, Jayanthi Shastri, Nupur Mukherjee, Kiran Munne, Vikrant M. Bhor, Taruna Madan, Vainav Patel

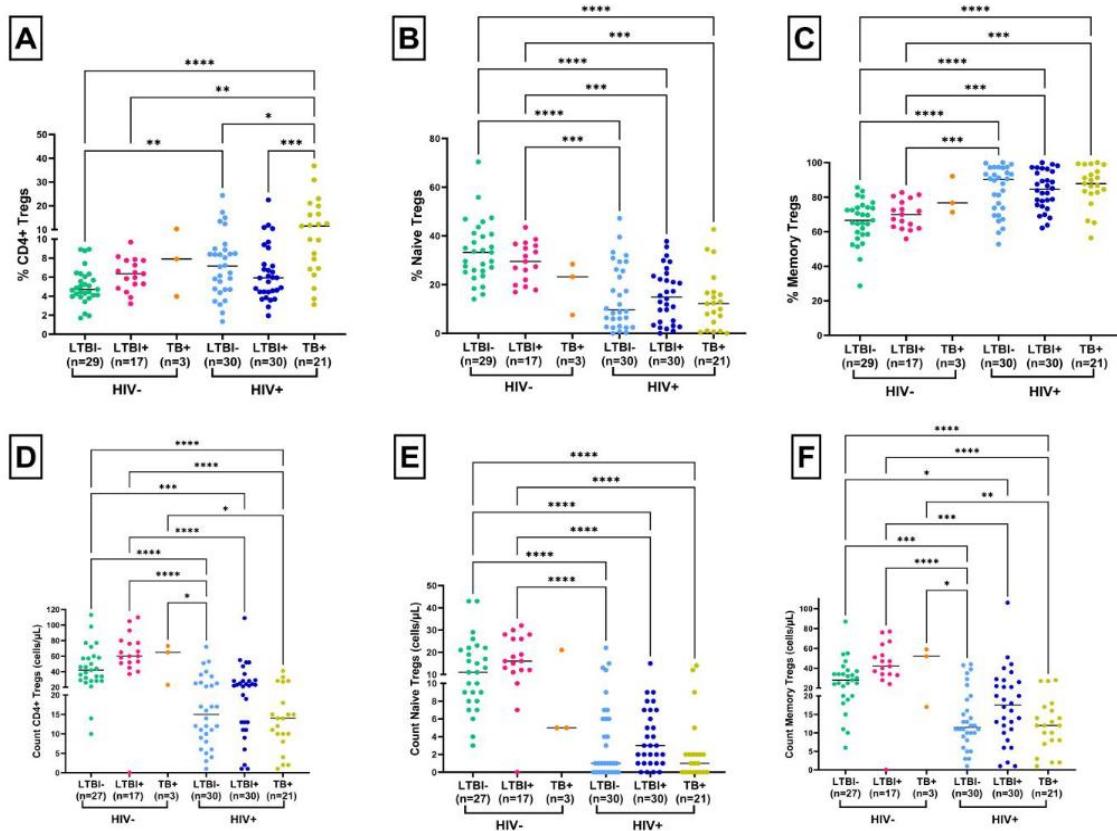
ICMR-National Institute for Research in Reproductive and Child Health, Mumbai

Background: Active TB infection is the leading cause of morbidity and mortality in people living with HIV (PLHIV). As CD4+ T cells are responsible for protection against MTb infection, depletion of these cells during HIV co-infection leads to reactivation of TB leading to detrimental effects in individuals with LTBI. Our study undertook the delineation of unique systemic pathogenic signatures associated with LTBI and active TB in the context of treatment naïve HIV-1 infection towards developing strategies to minimize morbidity and mortality caused by TB reactivation.

Method: Fifty HIV sero-negative and 81 HIV-1 infected ART naïve participants were recruited from tertiary care hospitals in Mumbai and were further stratified as LTBI+ and Active TB+ based on Interferon Gamma Release Assay (IGRA) and clinical confirmation respectively. Flow-cytometry was employed for immunophenotyping of various CD4+ T cell subsets and functionality and further plasma analytes were measured by ELISA and LUMINEX assays in-order to examine systemic immune mediators.

Result: We observed significantly lower absolute CD4 count and CD4/CD8 ratio in the HTB+ group compared to HLTBI+ group. Conversely, viremia was higher in the HTB+ group compared to both the HLTBI+ and HLTBI- groups suggesting that co-infection with active TB favoured HIV-1 viral replication and concomitantly, increased pathology in terms of lowered CD4 counts and CD4:CD8 ratios. With low levels of activation (HLADR+ CD38+) and PD-1 expression accompanied with decreased T regulatory cells during HLTBI+ state, we report on diminished HIV pathogenicity when compared to HIV+ individuals either with Active TB or without any co-infection. These results were supported with high levels of plasma IL-10 and IL-17A suggestive of immunomodulatory environment by LTBI confounding the effect of HIV.

Conclusion: Our results revealed unique signature highlighting a possible regulatory mechanism conferred by latent MTb which may provide strategies to mitigate HIV disease progression.



Frequency and counts of Regulatory T cells and its subsets. Frequency of CD4+ (A) Total Tregs (B) Naïve Tregs (C) Memory Tregs and absolute counts of (D) Total Tregs (E) Naïve Tregs (F) Memory Tregs within different HIV seronegative and HIV-1 positive groups. Comparisons between groups were calculated by Kruskal-Wallis one-way ANOVA non-parametric test, (*p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001).

16th Annual Conference & Workshops TCS 2024

Oral abstracts (Basic):

BO-02: Systemic and HCMV specific cellular immune signatures associated with pregnancy outcomes and congenital transmission: a prospective cohort study.

Harsha Chandrashekhar Palav, Shilpa Velhal, Tejaswini Pandey, Sayantani Ghosh, Sapna Yadav, Amit Kumar Singh, Varsha Kalsurkar, Devika Hande, Varsha Padwal, Forum Shah, Ira Shah, Purnima Satoskar, Vikrant Bhor* and Vainav Patel*

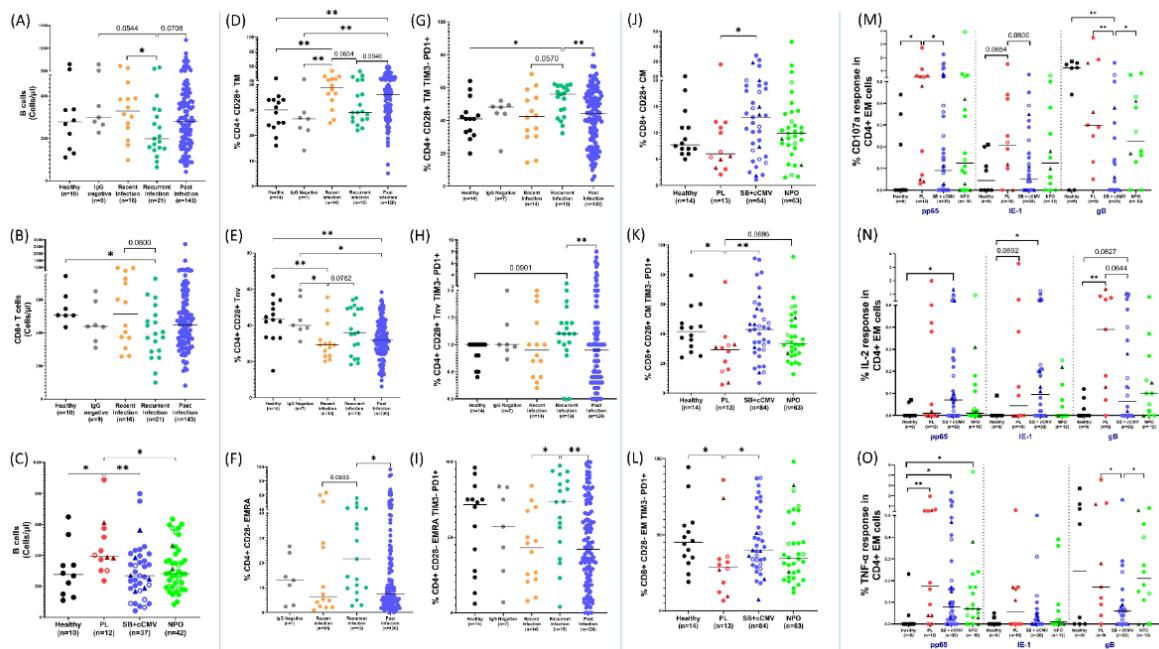
ICMR-National Institute for Research in Reproductive and Child Health, Mumbai

Introduction: Human Cytomegalovirus (HCMV) is a major cause of congenital infections (cCMV) worldwide, with over 90% seroprevalence among Indian women of reproductive age. Research on HCMV-specific immunity during pregnancy, especially regarding virus-specific T cells critical for viral clearance, is limited. This prospective study used multiparametric flow cytometry to investigate the impact of maternal HCMV infection and immune profiles on pregnancy outcomes.

Methodology: Pregnant women, with (n=45) and without Bad Obstetric History (n=38), were followed until delivery. HCMV infection status was assessed and pregnancy outcomes were recorded. cCMV was detected in neonatal saliva and cord blood using nested-PCR. Systemic cellular immune responses were analyzed alongside HCMV anamnestic T-cell responses against pp65, IE1, and gB antigens, assessing degranulation-(CD107a), chemokine production-(MIP-1 β), and Th1 cytokine production-(IFN- γ , TNF- α , IL-2) via intracellular cytokine staining and flow cytometry.

Results: We observed decrease in absolute counts of B-cells, CD8+ T-cells, and in frequency of CD4+ TM cells, along with increase in CD4+ EMRA, naïve cells, and PD1 expression across CD4+ T-cell subsets in recurrent infection group compared to recent infections. B-cell counts were higher in pregnancy loss group. In symptomatic birth and cCMV group, we noted increased activation of CD4+ CM cells, PD1 expression in CD4+ subsets, higher CD8+ EMRA, and increased activation and PD1 expression in CD8 subsets. Recurrent HCMV infections showed increased degranulation, IFN- γ and TNF- α production within CD4+ CM cells, and MIP-1 β within non-naïve CD8+ T-cells compared to recent infections. Additionally, increased degranulation, IFN- γ and TNF- α production were noted in non-naïve CD4+ T cells in pregnancy loss group, while the symptomatic birth and cCMV group exhibited reduced cytolytic responses, lower IFN- γ and TNF- α production, and higher IL-2 levels within non-naïve CD4+ T-cells.

Conclusion: Our research underscores putative systemic and HCMV specific maternal immune correlates for pregnancy loss and symptomatic birth along with cCMV.



Systemic cellular and HCMV specific recall T cell response. (A-C) Absolute count of B cells and CD8+ T cells within (A, B) HCMV infection status groups and (c) pregnancy outcome groups. Frequency of different CD4 and CD8 T cell subsets along with PD1 expression within (D-I) HCMV infection status groups and (J-L) pregnancy outcome groups. (M-O) HCMV specific recall T cell response upon stimulation with pp65, IE-1 and gB within pregnancy outcome groups.

16th Annual Conference & Workshops TCS 2024

Oral abstracts (Basic):

BO-03: Immune monitoring based protective signatures in survivors of the 2023 Nipah outbreak in Kerala, India.

Rima R. Sahay, Harsha C Palav, Anita M. Shete, Deepak Y. Patil, Sreelekshmy Mohandas, Chandni Radhakrishnan, Shihabudheen P, Anoop Kumar AS, Anitha Puduvail Moorkoth, Nandan Mohite, Pranay Gurav, Rajlaxmi Jain, Yash Joshi, Lathika Velichapati Ramakrishnan, Nivedita Gupta, Vainav Patel, Pragya D. Yadav

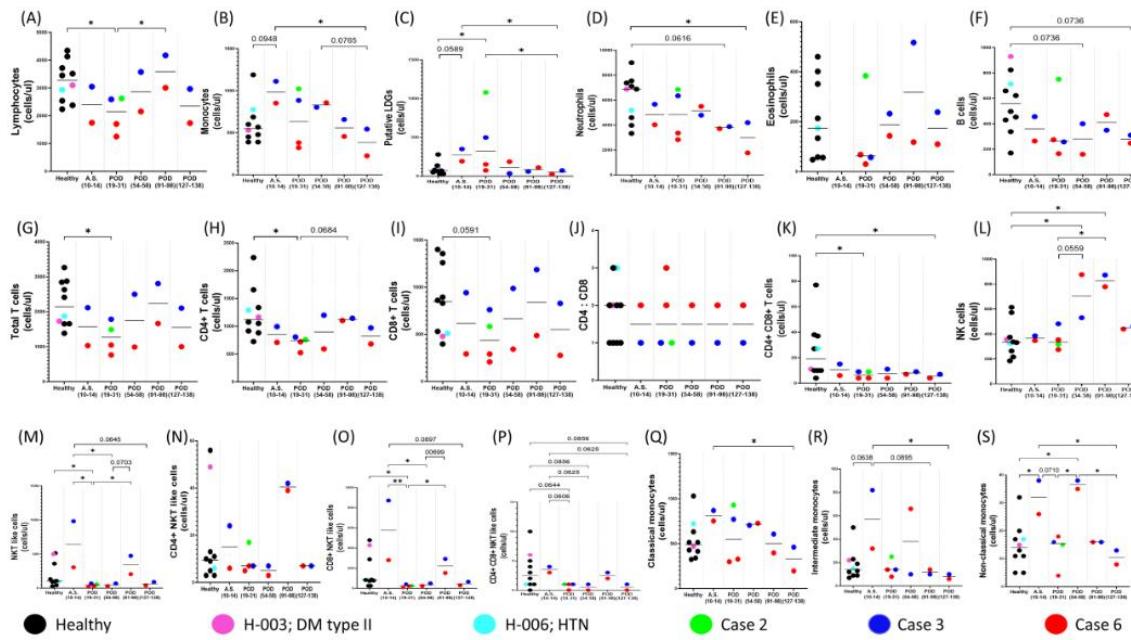
ICMR-National Institute for Research in Reproductive and Child Health, Mumbai

Background: Nipah Virus (NiV) is an emerging zoonotic pathogen of concern causing disease with significant morbidity and mortality. To enhance our understanding of NiV pathogenesis and to improve therapeutic and diagnostic approaches, it is crucial to analyze the immune profiles of survivors. This study focused on the immune characteristics of individuals (n=3), showing distinct clinical disease progression, who survived the 2023 NiV outbreak in Kerala, India, aiming to identify the immune mechanisms that confer protection against the virus.

Methods: We employed multiparametric flow cytometry to quantify and compare immune cell populations between survivors (up to four months post-infection) and healthy controls. Statistical analyses were performed to explore correlations between immune profiles and clinical outcomes.

Results: The study revealed several key immune signatures common to all three cases were: A previously undescribed persistent lymphopenia, particularly affecting CD4+ T cells, including an expansion of memory Tregs (regulatory T cells); trends indicative of global leukopenic modulation in monocytes and granulocytes, including an expansion of putatively immunosuppressive low-density granulocytes (LDGs) recently described in severe COVID-19 cases; altered mucosal homing with respect to integrin beta-7 (ITGB7) expressing immune cell subsets; increased mobilization of activated T cells (CD4+ and CD8+) and plasmablasts in the acute symptomatic phase of infection. Comparative analysis of clinical presentation and outcome suggested lower initial viremia, increased activated T-cell responses, expanded plasmablasts, and restoration of ITGB7-expressing CD8+ T cells were associated with milder NiV disease.

Discussion: This longitudinal study identifies potential protective immune signatures linked to milder NiV disease and supports the exploration of immunotherapeutic options, such as monoclonal antibodies, to mitigate early viremia and improve disease outcomes.



Ex-vivo Immune monitoring by evaluating absolute counts of Leukocytes: T cells, B cells, NK cells and Monocytes between healthy and NiV infected individuals at different phases of infection based on point of detection (POD).

(A) Lymphocytes (B) Monocytes (C) Putative low density granulocytes (D) Neutrophils (E) Eosinophils (F) B cells (G) Total T cells, CD3+ (H) CD4+ T cells (I) CD8+ T cells (J) CD4 CD8 ratio (K) CD4+ CD8+ T cells (L) NK cells (M) NKT like cells (N) CD4+ NKT like cells (O) CD8+ NKT like cells (P) CD4+ CD8+ NKT like cells (Q) Classical monocytes (R) Intermediate monocytes and (S) Non-classical monocytes. A.S.: Acute Symptomatic. The color codes (Green, blue and red) represents NiV cases. In the NiV infected group all data points, including follow-up from patients are plotted (from POD 10-58, as available). While the color codes of control group with apparently healthy individuals are pink (Controlled Type II Diabetes mellitus) cyan blue (Controlled hypertension); and black (With no co-morbidities). Statistical significance was calculated by non-parametric one-way ANOVA (Kruskal-Wallis test); *, p < 0.05; **, p < 0.01.

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Oral abstracts (Basic):

BO-04: In depth immunological and viral analysis of the HIV-1C putative reservoir in an Indian cohort.

Snehal Kaginkar, Shilpa Bhowmick, Nandini Kasarpalkar, Sharad Bhagat, Jyoti Sutar, Ranajoy Mullick, Priyanka Jayal, Pranay Gurav, Sapna Yadav, Satyajit Musale, Tejaswini Pandey, Namrata Neman, Ashashree Sahoo, Varsha Padwal, Amit Kumar Singh, Shilpa Velhal, Sayantani Ghosh, Varsha Kalsulkar, Gita Nataraj, Kavita Joshi, Sachee Agrawal, Sushma Gaikwad, Jayanthi Shastri, Jayanta Bhattacharya, Vainav Patel

ICMR-National Institute for Research in Reproductive and Child Health, Mumbai

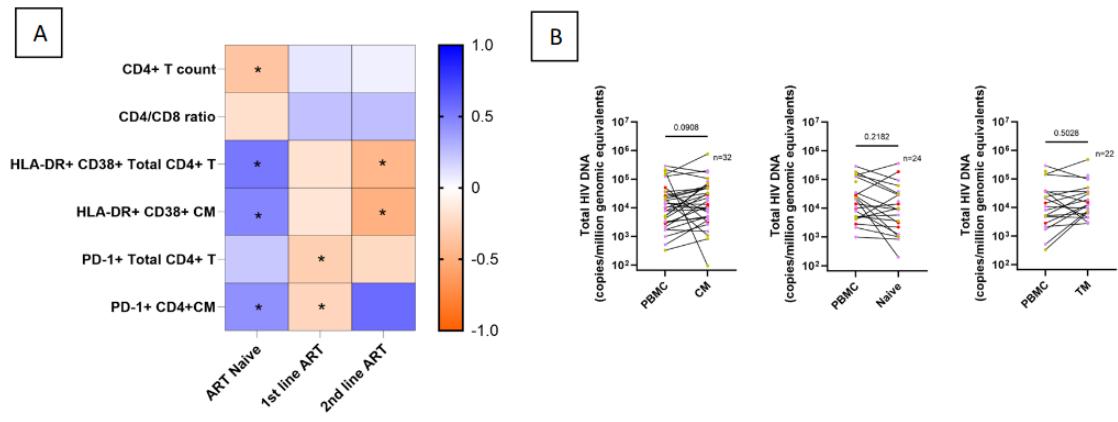
Introduction: HIV transmission and disease manifestation has been reduced significantly due to successful roll out of Anti-Retroviral Therapy (ART). However, HIV persists in reservoirs which could contribute to rebound viremia or emergence of drug-resistant viruses. Also, residual low-level viremia with accompanying viral evolution can cause chronic activation and exhaustion of T cells resulting in seeding/ proliferation of existing reservoirs.

Methodology: Study participants were recruited from tertiary care hospitals, Mumbai. Participants were further grouped as per their therapy status. T cell functionality was evaluated using flow cytometry. HIV proviral load was estimated from sorted CD4+T cell subsets and near full length amplicons were generated followed by NGS. Drug resistance mutation (DRM) analysis was performed using Stanford HIV drug resistance database. Neutralization efficiency of broadly neutralizing antibodies (bnAbs) was tested against patients' env derived pseudoviruses in vitro.

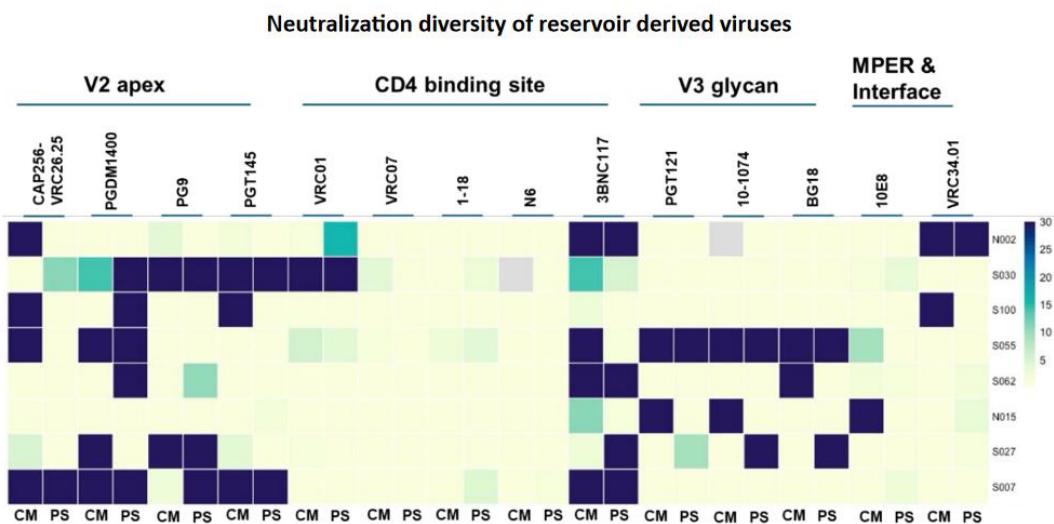
Result: Functional properties of putative reservoirs (CD4+ T cells) are correlated with each other and with reservoir burden (proviral load) in ART naive conditions where dysfunctional activation and PD-1 expression are observed. However, ART mediated partial restoration of this dysfunction does not result in appreciable reduction of reservoir. The post ART proviral burden was persistent post ART and found to be enriched in circulating CD4+ central memory (CM) T cells. Most of the reservoir derived envelopes were found to be sensitive to CD4 binding site directed bnAbs but displayed disparate neutralization profiles across compartments. DRM analysis showed presence of primary resistance to the current therapy regimen.

Conclusion: Our data highlighted disparate immune profiles of T cells even in ART responding individuals. It also suggests that proposed bnAbs combination for circulating viruses would need modification to target reservoir viruses. Viral surveillance, both prior to and post ART must include diversity of circulating CD4+ CM resident proviruses in order to optimize and rationally guide bnAb based interventions.

Effect of Anti-Retroviral therapy on association between reservoir size and functional markers



Association between proviral loads and functional markers. (A) Correlation between PBMC proviral load in ART naïve, 1st line ART receiving, 2nd line ART receiving groups (as columns) with CD4+ cell count, CD4/CD8 ratio, HLA-DR+ CD38+ co-expression in total and CM CD4+ T subsets and PD-1 expression in total and CM CD4+ T subsets (as rows) displayed in the heatmap. Orange indicates negative correlation and purple indicates positive correlation. (B) Paired analysis of proviral DNA enrichment (from left to right) in CM, naïve and TM CD4+ T subsets compared to PBMC. Statistical analysis was done using Wilcoxon t test



In vitro validation of bnAb based neutralization of env pseudo-viruses from individuals. 14 bnAbs were tested for their efficiency to neutralise pseudoviruses generated from 8 participants. bnAbs are classified as per their target epitopes Colour gradation low to high represents resistance to bnAbs in central memory compartment Vs PS (PBMC) compartment.

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Oral abstracts (Basic):

BO-05: Assessment of SARS-CoV-2 specific immunity in ChAdOx1 nCoV-19 vaccinated subjects with type 2 diabetes.

Swati, Pinaki Dutta, Preetam Basak, Sanjay Bhadada, Madhu Gupta, Mini P Singh, Naresh Sachdeva*

PGIMER, Chandigarh

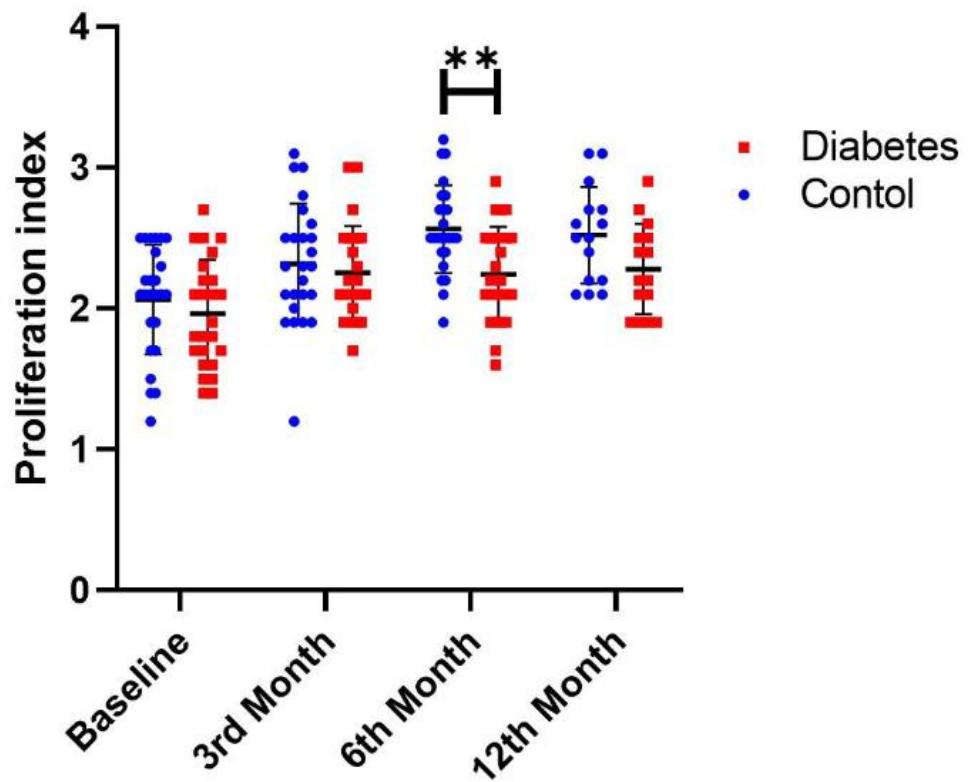
Background: We and others have reported impaired antibody responses, post-COVID-19 infection, and vaccination in type 2 diabetes (T2D). This study aimed to characterize SARS-CoV-2 specific immunity post-ChAdOx1 nCoV-19 vaccination in T2D subjects.

Methods: We recruited 55 T2D and 60 healthy control (HC) subjects and monitored their immunological parameters at baseline, 3rd, 6th, and 12th months. All subjects underwent HLA typing and the frequency of SARS-CoV-2 epitope-specific CD8+ T cells was determined by flow cytometry (FC) using MHC-I dextramers. The memory T cell responses were assessed by T cell proliferation and estimation of intra-cellular (TNF- α , IFN- γ) and extra-cellular (IL-2, IL-4, IL-6, IL-10, IFN- γ , TGF- β) cytokines following in-vitro stimulation of PBMCs with SARS-CoV-2 peptides (SP). The anti-S antibody titers (Ab-S) were estimated by ECLIA. The frequency of memory B cells (CD19+CD27+), plasmablasts (CD19+CD27hiCD38hi), and plasma cells (CD38hi CD138+) were determined by FC.

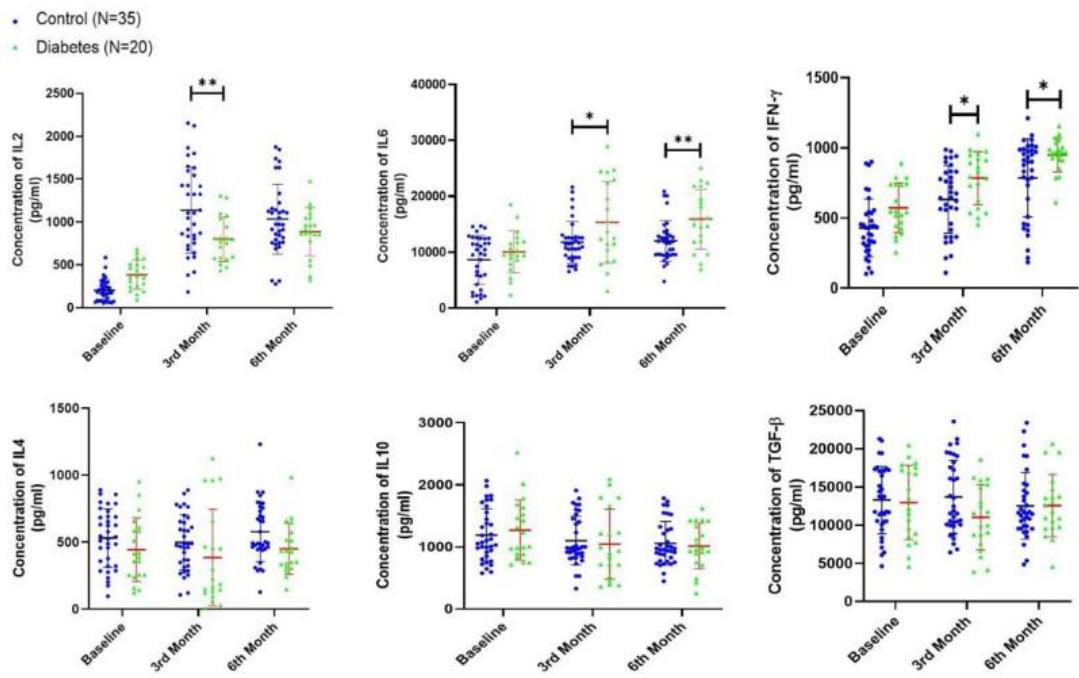
Results: The vaccinated subjects harbored CD8+ T cells specific to epitopes of spike protein. The T2D subjects had lower SARS-CoV-2-specific lymphocyte proliferation indices at 6th month [T2D:2.2 (2-2.5) vs. HC:2.5 (2.4-2.7); p=0.008]. Similarly, IL-2 levels (pg/ml) were lower in T2D subjects at 3rd month [T2D:787.9 (580.8-1018); HC:1105 (754.5-1546); p=0.0013]. The levels of IL-6 (pg/ml) were higher in T2D subjects at 3rd [T2D:13168 (8972-22884) vs HC:10653 (8576-12500); p=<0.05] and 6th month [T2D:15471 (11925-21319) vs HC:11853 (9565-13256); p<0.05]. The levels of IFN- γ (pg/ml) were also higher in T2D subjects at 3rd [T2D:814.1 (584.5-944.2) vs HC:645.2(414.9-830.5); p<0.05] and 6th month [T2D:967.4 (913.5-1027) vs HC:899.2 (507.7-986.3); p<0.05]. The frequency of CD8+ T cells secreting IFN- γ was higher in HC subjects at 6th [T2D:10.63 (8.2-13.65) vs. HC:20.37 (14.97-23.67); p<0.05] and 12th month [T2D:6.7 (5.9-8.73) vs. HC:18.23 (12.63-23.28); p<0.05]. Similarly, frequency of CD8+ T cells secreting TNF- α was higher in HC at 12th month [T2D:11.6 (8.7-14.6) vs. HC:20.43 (13.27-22.15); p<0.05]. The Ab-S titres were higher in T2D subjects at 6th month [T2D:6161 U/ml (2238-12310) vs HC:3443 U/ml (1370-5704); p<0.05)]. However, the relative percentage of memory B, plasma, and plasmablast cells was lower in T2D subjects.

Conclusion: T2D impairs vaccine-induced SARS-CoV-2 specific immunological memory responses.

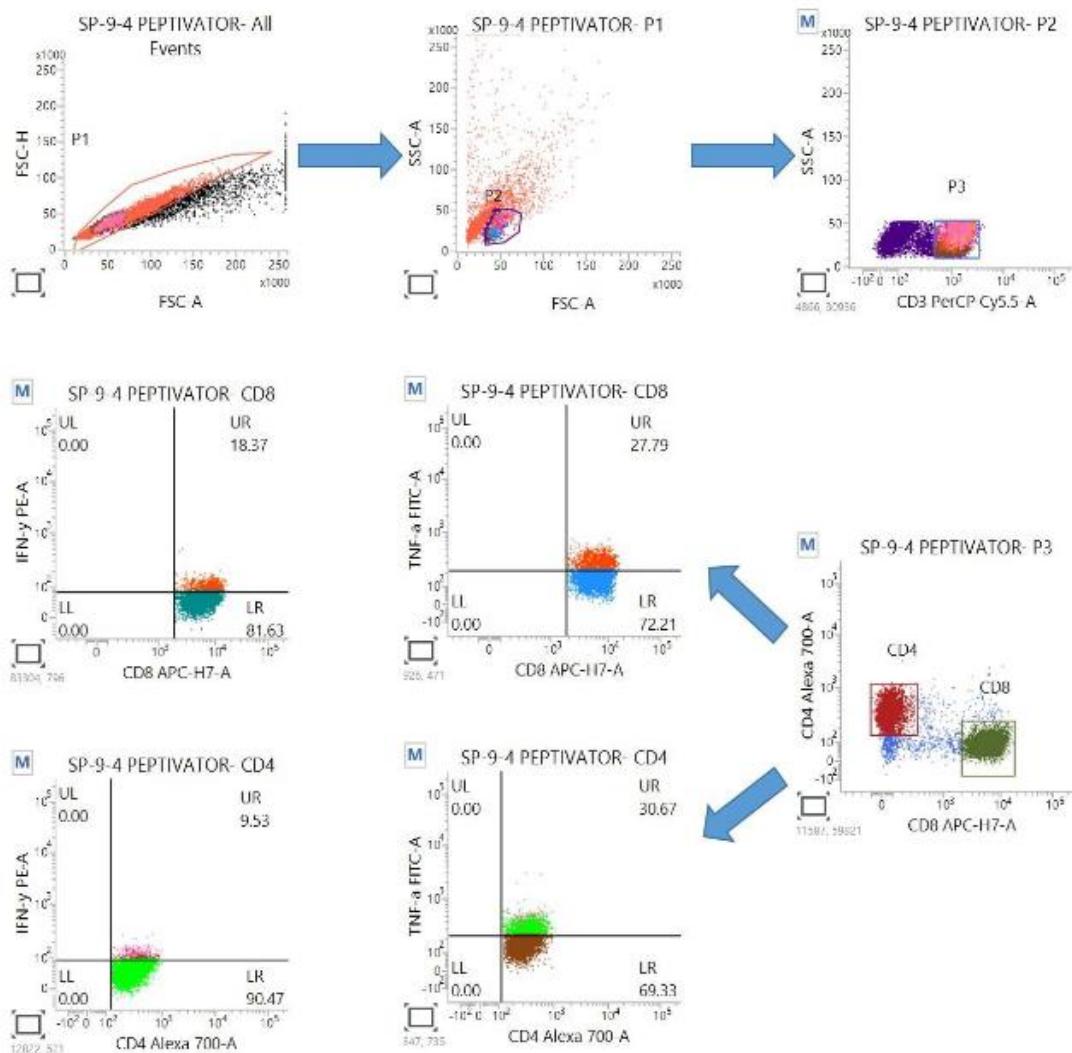
Lymphocyte proliferation indices



Extracellular cytokine analysis

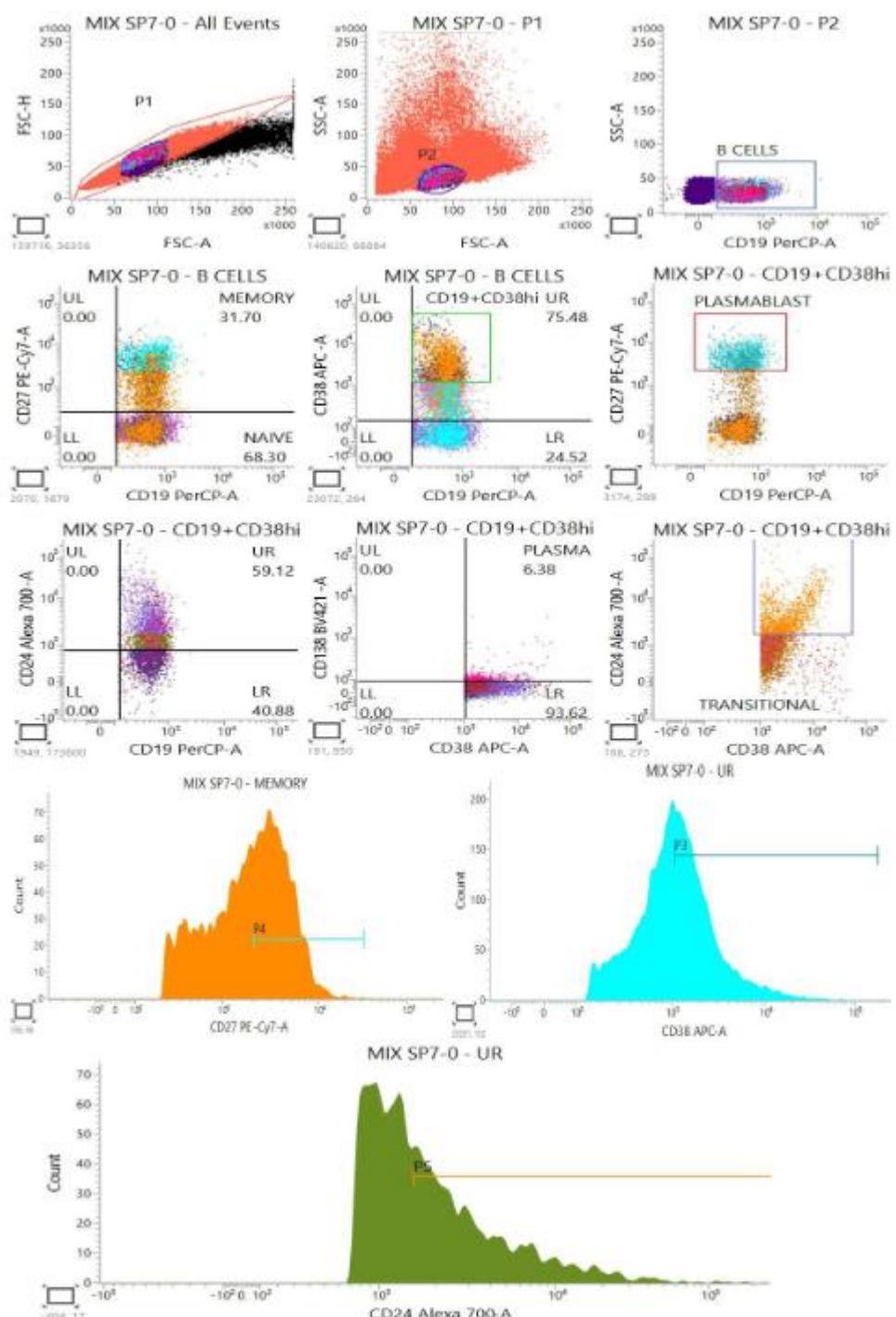


Intracellular Cytokine analysis



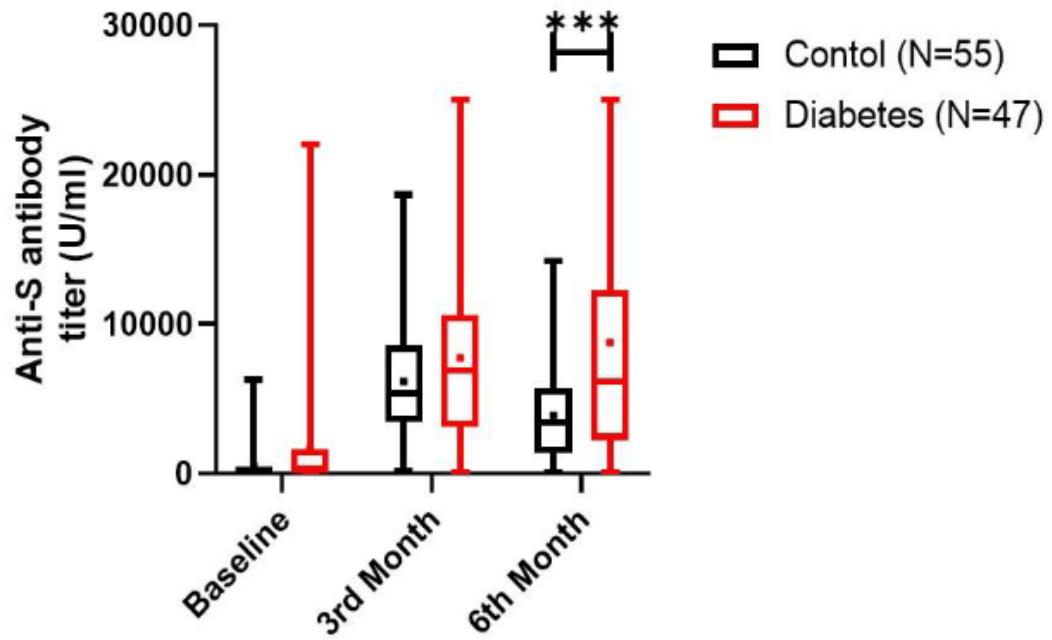
Intracellular Cytokine analysis: Representative plots showing expression of intracellular cytokines (IFN- γ and TNF- α) within CD4+ and CD8+ T cells separately. Appropriate FMO controls were used to set gates.

Peripheral memory B cell frequency



Peripheral B cell frequency: Immunophenotyping of different B cell subsets [CD19+ (Pan B-cell marker), CD19+CD27- (naïve), CD19+CD27+ (memory), CD19+CD38hi CD24hi (transitional), CD19+CD27hiCD38hi (plasmablasts) and CD38hi CD138+ (plasma)].

Anti-SARS-CoV-2 antibody titres



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POSTER ABSTRACTS (CLINICAL)

CP-01: The Thyroid Enigma: Duel of the Shadows - “ A RARE CASE REPORT”

Dr. Abhishek Kamble, Dr Ravindra Shinde, Dr Sandeep Yadav.

D.Y Patil Medical college, Kolhapur

Background: Collision tumors are exceptionally rare and can feature components with diverse behaviours, treatment strategies and prognoses. We present an unprecedented case involving an aggressive thyroid collision tumor that includes widely invasive oncocytic carcinoma (OC) and poorly differentiated thyroid carcinoma (PDTC) which clinically and radiologically showed benign but was subsequently diagnosed as a collision tumor with aggressive characteristics. Thyroid collision tumors are extremely infrequent and early detection can exhibit better prognosis.

Case Report: A 65 year-old male came with complaints of anterior left side neck swelling over past 1 year. Patient had a history of smoking. Laboratory evaluation showed euthyroid thyroid profile(T3, T4 and TSH were within normal limits). Family history of goitre was absent. Radiological investigation (USG) were suggestive of colloid goitre. Hence, Left hemithyroidectomy was done and sent for histopathological examination which revealed aggressive collision tumor of Oncocytic cell carcinoma and poorly differentiated thyroid carcinoma. Later it was confirmed by immunohistochemistry.

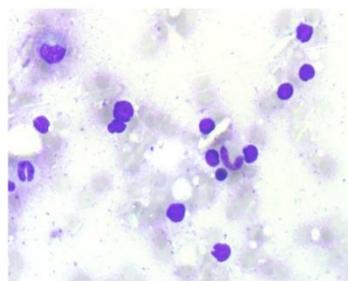
Conclusion: Thyroid collision tumors are extraordinarily uncommon and present considerable difficulties in both diagnosis and treatment. This is very unique case of a thyroid collision tumor featuring histological patterns of oncocytic carcinoma and a potentially progressive component of poorly differentiated thyroid carcinoma (PDTC).

CP-02: A rare presentation of CALLA Positive B Acute Lymphoblastic Leukemia in association with Storage disorder (Gaucher's)

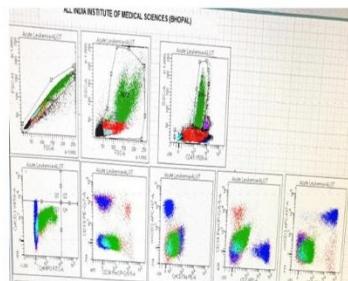
E. Jayashankar, Shivani Joshi, Jai Kumar Chaurasia, Ashwani Tandon, Garima Goel, Vaishali Walke, Narendra Kumar Choudary, Bhavana Dhingra, All India Institute of Medical Sciences Bhopal

Abstract: This is a case of one year old male child, who presented with pediatrics OPD with complaints of fever, hepatosplenomegaly, and pain abdomen. No lymphadenopathy. On hematological investigations, he was diagnosed with, acute leukemia (22% Blasts). These blasts are 1 to 1 ½ times the size of small mature lymphocytes, with increased N:C ratio, scant to indistinct cytoplasm with no granules, & No Auer rods. Bone marrow aspiration smears revealed 25% blasts, of similar morphology. The flow report, shows 30% of CD45 dim population of blasts, positive for CD10, CD19, CD20, CD34, CD38, CD58 and HLA DR. other markers, CD117, TdT, MPO, and other T Cell markers were Negative in the gated population, and the diagnosis was confirmed with CD10 positive B ALL. Trephine sections was shown the presence of the histiocytes, which were having abundant cytoplasm (crumpled tissue paper appearance), central to peripherally placed nucleus, arranged in sheets and intermittent pattern Hence, we reported the trephine section as Marrow involvement of CALLA positive ALL, in association with Storage disorder (Gaucher's). The patient blood sample is submitted for enzyme studies, to further confirm with enzyme deficiency. Gaucher's disease is a rare and autosomal recessive sphingolipidosis, and most common lysosomal storage disorder. A congenital deficiency of beta-glucocerebrosidase results in the accumulation of glucocerebroside in the lysosomes of macrophages (Gaucher cells), giving the characteristic appearance of crumpled tissue paper appearance. The accumulation of these macrophages results in enlargement of the organs involved. Most common organs involved are bone marrow, spleen, liver. Hence the patient usually presents with organomegaly. The association of acute leukemia and Gaucher's was first reported in 1961 by Gelfand and Gribroff. There is altered cellular and marrow microenvironment, resulting in leukemia. References: 1. Mishra S, Hajra S, Arathi K, Gupta AK. Gaucher's disease with acute lymphoblastic leukemia: A rare co-occurrence. J Hematol Allied Sci 2022;2:96-8. 2. Mistry PK, Taddei T, vom Dahl S, Rosenbloom BE. Gaucher disease and malignancy: A model for cancer pathogenesis in an inborn error of metabolism. Crit Rev Oncog 2013;18:235-46. 3. Cobert GM, Darbyshire PJ, Besley GT, Parker AC. Adult Gaucher disease in association with acute leukemia. Postgrad Med J 1977;63:899-900. 4. Watek M, Piktel E, Wollny T, Durnas B, Fiedoruk K, Lech-Maranda E, et al. Defective sphingolipids metabolism and tumor associated macrophages as the possible links between gaucher disease and blood cancer development. Int J Mol Sci 2019;20:843.

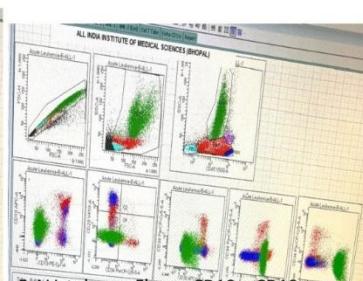
Image:



a. Lymphoblasts on Giemsa 100x

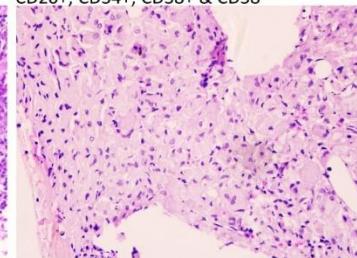
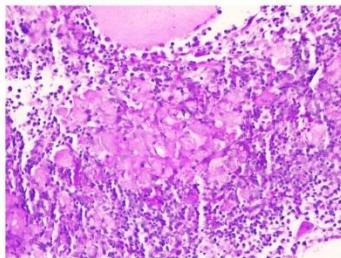
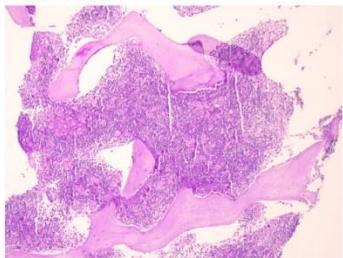


b. ALOT showing CD19+, & CD34+



B. ALL tube on Flow - CD10+, CD19+,

CD20+, CD34+, CD38+ & CD58



d,e (PAS), f.(H&E) Trehine section – sheets immature cells admixed with histiocytes (Gaucher cells) in nodules and in sheets

CP-03: Autoimmune Lymphoproliferative Syndrome: Overcoming Diagnostic Hurdles in a Tertiary Care Centre in Eastern India

Dr Tanya Prasad, Dr Surabhi, Dr Swarnim, Dr Chandra Mohan Kumar

AllIMS Patna

Autoimmune Lymphoproliferative Syndrome (ALPS) affects lymphocyte regulation due to defects in the FAS apoptotic pathway. It is marked by non-malignant lymphoproliferation, autoimmune disorders—especially multilineage cytopenias—and an elevated risk of B-cell lymphoma. Most patients harbour heterozygous germline mutations in the gene for the TNF receptor-family member Fas (CD 95, Apo-1) which are inherited in an autosomal dominant fashion. Somatic Fas mutations are the second most common genetic etiology of ALPS. We

encountered three cases at our center with recurring skin issues, upper respiratory tract symptoms, and prolonged fever. Upon examination, these patients have multiple clusters of enlarged lymph nodes, splenomegaly, and persistent, unexplained cytopenias. Flow cytometry of peripheral blood samples revealed a substantial population of double-negative CD3+ T-lymphocytes. According to the updated diagnostic criteria, all the cases fulfilled the criteria for a "Probable Diagnosis" of ALPS. However due to the financial constraint further

genetic workup could not be done to confirm the mutations. The children were started on immunosuppressants which led to symptomatic relief.

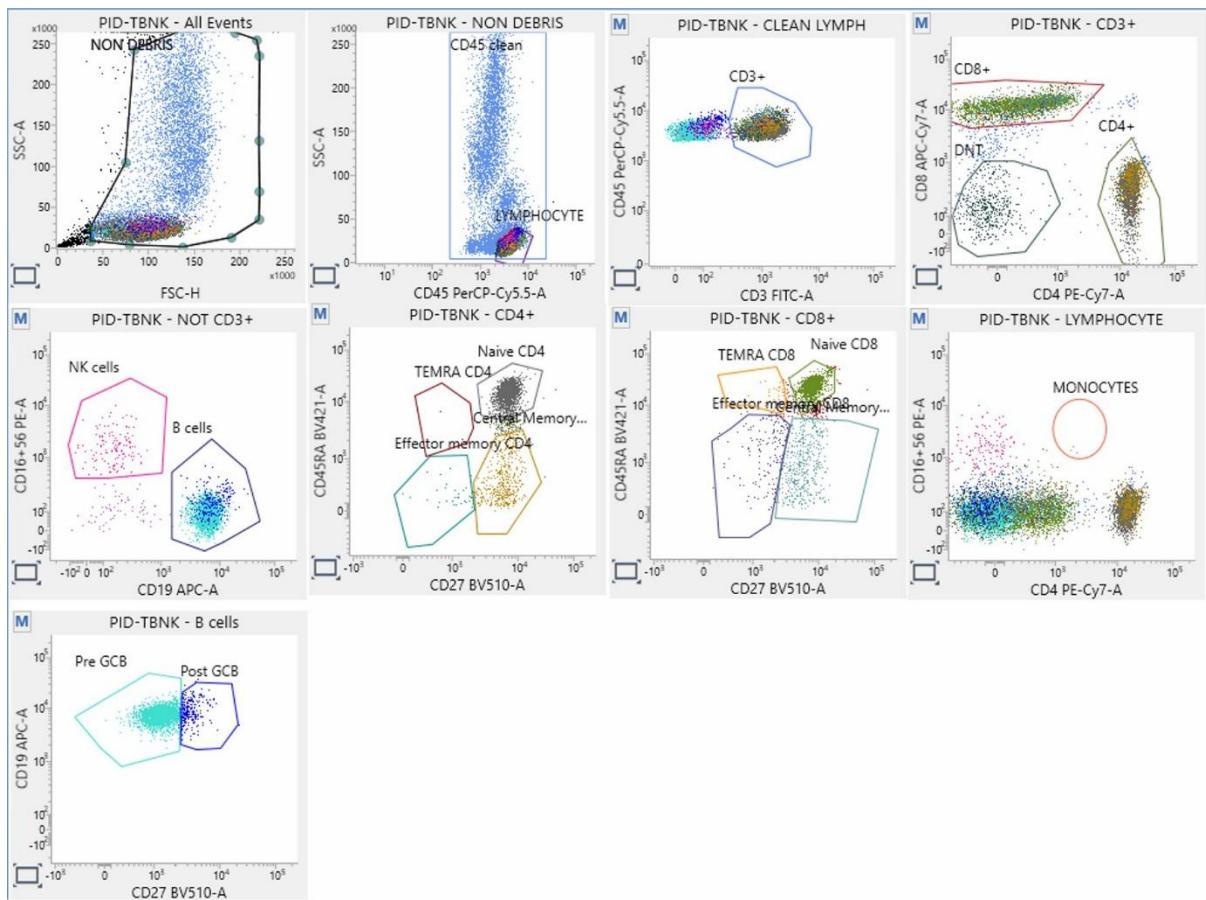
CP-04: Beyond TBNK subsets in Inborn errors of immunity (IEI) – Value of a modified 8 colour lymphocyte subset panel for routine screening, as a low-cost alternative to the Euroflow PIDOT approach.

Dr Ananthvikas Jayaram

Neuberg Anand Reference Laboratory, Bangalore

Abstract

Background: SCID is one of the commoner IEIs, classically defined by absent T cells. However, variants of SCID are increasingly being discovered with decreased thymic output but preserved T cell counts. Early diagnosis in SCID is imperative as early treatment is directly linked to improved outcomes. We share data on a modified lymphocyte subset panel used to screen over 400 children for SCID, and highlight its advantage over a 6 colour TBNK panel in the context of IEIs. **Method:** The 6 colour TBNK cocktail from BD Biosciences was expanded using drop-ins of CD45RA BV421 and CD27 BV510. CD4 and CD8 T cells were further subclassified to Naïve and memory subsets using a CD45RA vs CD27 plot, and B cells were subclassified into Naïve and Memory B cells. All samples were processed on EDTA Whole blood using a stain-lyse-no-wash protocol, and absolute counts calculated using a dual platform technique. Immunoglobulin estimation was performed on a Siemens nephelometer. **Results:** A total of 635 samples were processed, of which 417 samples were from children <5 years old, including 154 samples from children <1 year old. 29 samples (7%) showed a decreased CD4 Naïve T cell count, including 10 cases with absent CD4 Naïve cells. 7 of these showed a normal T cell count for age. CD8 Naïve T cells showed significant variations, including 55 cases with normal CD4 naïve cells showing decreased CD8 naïve cells. **Conclusion:** The clinical spectrum of SCID is varied, and a significant number of cases often show a mild decrease in T cell count. These may be missed if Naïve T cells are not estimated, leading to delays in diagnosis and poor outcome. Our data hopes to make a strong case to move toward a more comprehensive lymphocyte subset analysis while screening for PIDs than the routine TBNK assay.



CP-05: A Unique Confluence: Epidermoid Cyst and Secondary Massive Ovarian Edema in a Single Ovary.

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Background: Massive ovarian edema is an exceptionally rare benign condition characterized by substantial swelling of the ovary due to fluid accumulation and often associated with partial torsion of the ovarian pedicle. Epidermoid cysts of the ovary, although uncommon, are benign epithelial tumors which are asymptomatic and discovered incidentally during imaging studies or surgeries. This report highlights a unique case in which both massive ovarian edema and an epidermoid cyst were found within a single ovary, showcasing an unusual co-occurrence of these rare conditions.

Case Report: A 23-year-old unmarried female presented with abdominal pain and marginal symptoms. Laboratory tests showed no significant abnormalities. Ultrasound imaging revealed a bulky right ovary with a 30 ml fluid accumulation but no signs of abnormal vascularity or torsion of the pedicle. A cystectomy was performed, and the excised tissue was sent for histological examination, which later confirmed the presence of an epidermoid cyst with secondary massive ovarian edema.

Conclusion: This case underscores the rare but significant possibility of encountering both massive ovarian edema and an epidermoid cyst within a single ovary. While each condition is infrequent on its own and highlights the complexity of diagnosing and managing ovarian pathologies. The incidental finding of an epidermoid cyst amidst the presence of massive edema necessitates a thorough diagnostic approach, including detailed imaging and histological analysis. Awareness of this rare combination is crucial, particularly in cases where typical presentations may be misleading.

CP-06: Diagnostic utility of body fluid flow cytometry in patients with and without prior haematolymphoid malignancy.

Dr Vidisha Mahajan, Dr Khushbu Kasundra, Dr Shanaz Khodaiji

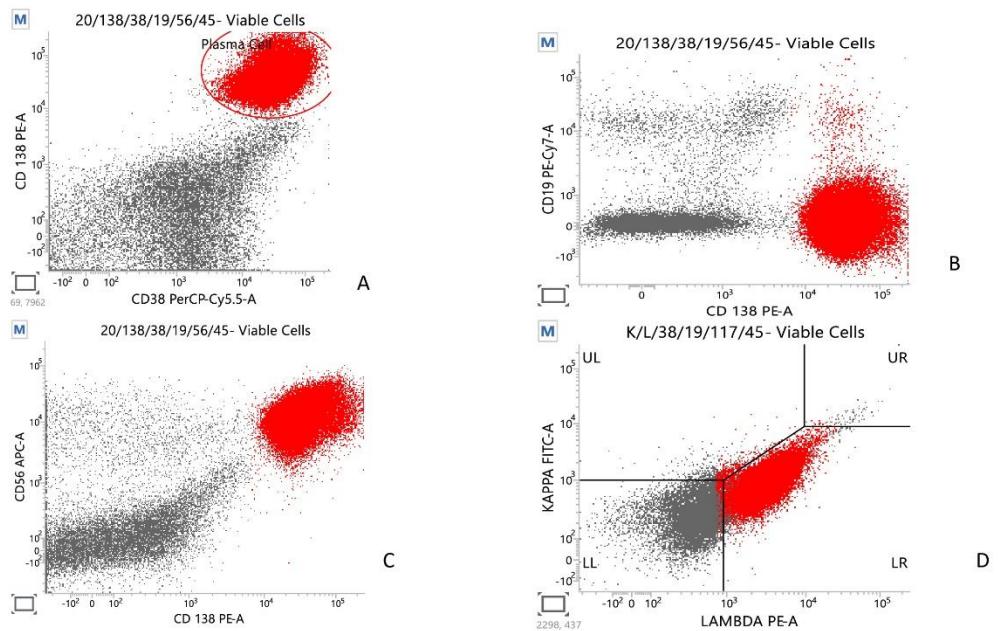
PD Hinduja Hospital and Medical Research Centre

Introduction: The occurrence of body cavity effusions is a symptom of a considerable pathologic process. The aetiology of effusions comprises a wide spectrum of diseases ranging from nonneoplastic ones to neoplastic ones. Malignant pleural effusions are common in patients with hematologic malignancies and they are reported to occur in up to 48% patients while involvement of the peritoneal and pericardial cavities seems to be less common. Up to 10% of malignant pleural effusions with a positive cytologic examination are due to non-Hodgkin lymphoma (NHL). In the vast majority of cases, serous effusions develop during the course of the disease, but rarely they may be the first presentation of a hematologic malignancy. The presence of cells of a hematologic malignancy in effusions is usually a sign of advanced and generalized disease.

Method: We reviewed cases of body fluids processed for flow cytometry in Haematology laboratory from January 2021 to January 2024. All the necessary details (specimen type, clinical history, previous diagnosis, demographic details) were recorded. In this retrospective study, total 54 cases were analyzed.

Result: A total of 54 body fluid samples were analysed over a span of 3 years. Thirty-eight of these patients had history of a haematolymphoid malignancy. Twenty-one cases of these known cases had fluid involvement by flow cytometry. Of the remaining 16 patients with no previous history of a haematolymphoid malignancy flow cytometry was able to pick up one case of myelomatous pleural effusion and cerebrospinal fluid (CSF) involvement by a Burkitt's lymphoma. Pleural fluid involvement was seen in 12 cases (22.2%) and CSF involvement was noted in 10 cases (18.5%). Body fluid flow cytometry revealed involvement in 13 cases of B cell neoplasms, 6 cases of T cell neoplasms, 2 cases of myeloid neoplasms and 1 case of acute leukaemia of ambiguous lineage.

Conclusion: Cytopathologists are regularly confronted with lymphocyte-rich effusions, and the definite decision of whether the lymphocytosis is of a purely reactive nature or a presentation of an indolent lymphoma may be an extremely difficult one based on morphology alone. Multiparametric flowcytometry has an important role in detecting body fluid involvement in haematolymphoid malignancy as it can detect even a few malignant cells that may be missed out by routine microscopy.



CP-07: Leukaemia and its variants

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St John's Medical College Hospital, Bangalore

Background :

Flowcytometry has known to be a method in differential diagnosis of chronic lymphoproliferative disorders. Subclassification of B cell Non Hodgkins Lymphomas is based on the classical immunophenotype proven by Flowcytometry on blood, bone marrow and lymph node samples.

Aim :

Evaluation of role of Immunophenotype by Flowcytometry in cases with lymphocytic leucocytosis for a diagnosis of Chronic Lymphocytic Leukaemia and its variants.

Materials and methods :

Retrospective review of cases 2020 to 2024. The samples (blood, bone marrow) were run on BD FACSCanto flowcytometer with panel of fluorescent antibodies including-CD45, CD20, CD23, CD10, CD79b, CD19, CD200, CD43,CD38,CD25,CD56,CD7,CD138,CD103,CD34,KAP PA and LAMDA in 3 tubes .

Results :

A total of 56 cases were included in the study. Mean Age at presentation was 65years (30-95years) Male:Female ratio was 1:1.5 Mean total count was 66,490 cells/ μ L (4,000 – 6,50,000 cells/ μ L) Mean Absolute lymphocyte count 52,507 cells/ μ L (2,000 – 5,80,000 cells/ μ L) Flowcytometry was done on 13 blood samples and 43 bone marrow samples. Immunophenotype consistent with Classical Chronic lymphocytic leukaemia (Positive for CD23+,CD200+,CD20,CD19+,CD5+) were seen in 46 cases (82%). Atypical chronic lymphocytic leukaemia immunophenotype with negative CD23 were seen in 10 cases (18%). These cases were followed up for a period of 0.5- 4 years. On follow up they had persistent lymphocytic leucocytosis in 4 cases, 2 case were loss to follow up and 4 cases showed normalisation of WBC count.

CP-08: A Rare Case of T-cell Acute Lymphoblastic Lymphoma Following Acute Myeloid Leukemia Remission: A Diagnostic Challenge

Dr. Sajna, Dr. Ann ThomaS; Mr. Binshad Ameer

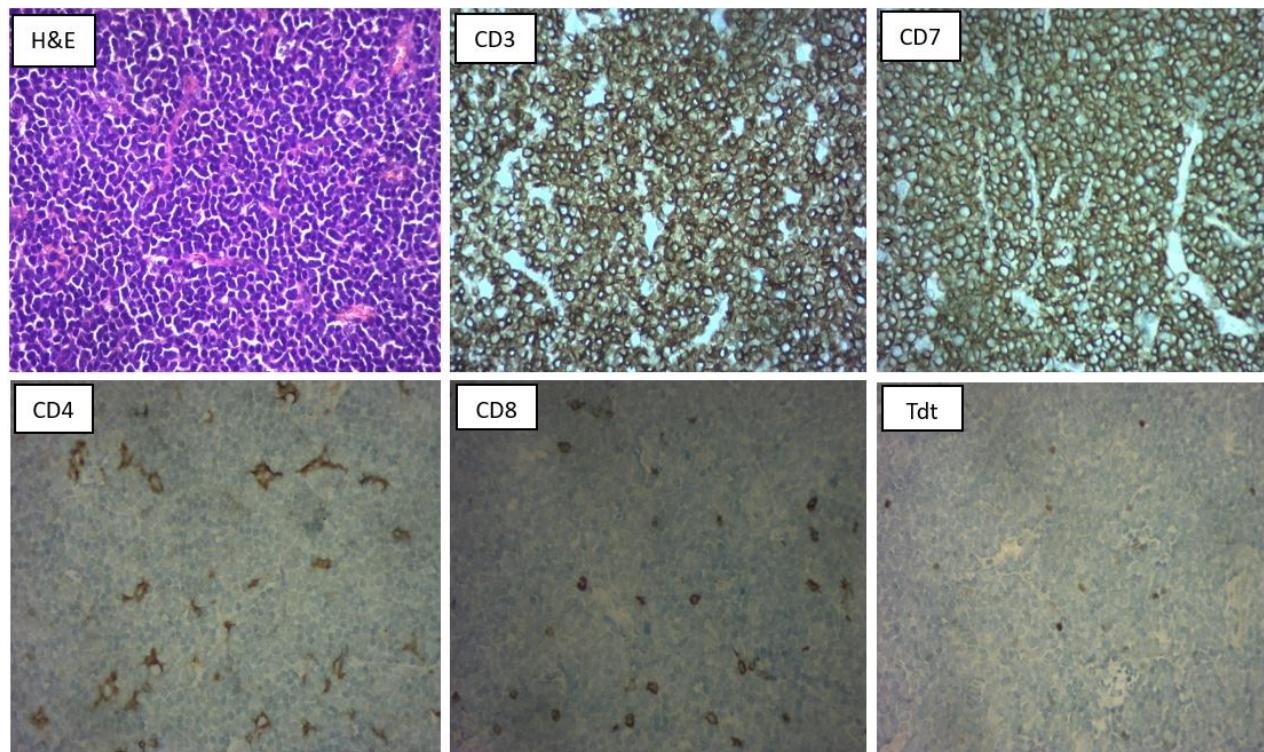
MVR Cancer Centre and Research Institute , Kozhikode, Kerala

Background: Acute Myeloid Leukemia (AML) survivors are at increased risk for developing secondary malignancies, among which T cell - Acute Lymphoblastic Leukemia / Lymphoma (T-ALL) is rarely described. Therapy-related ALL is a distinct entity with adverse genetic features and clinical outcomes, often presenting with complex diagnostic challenges.

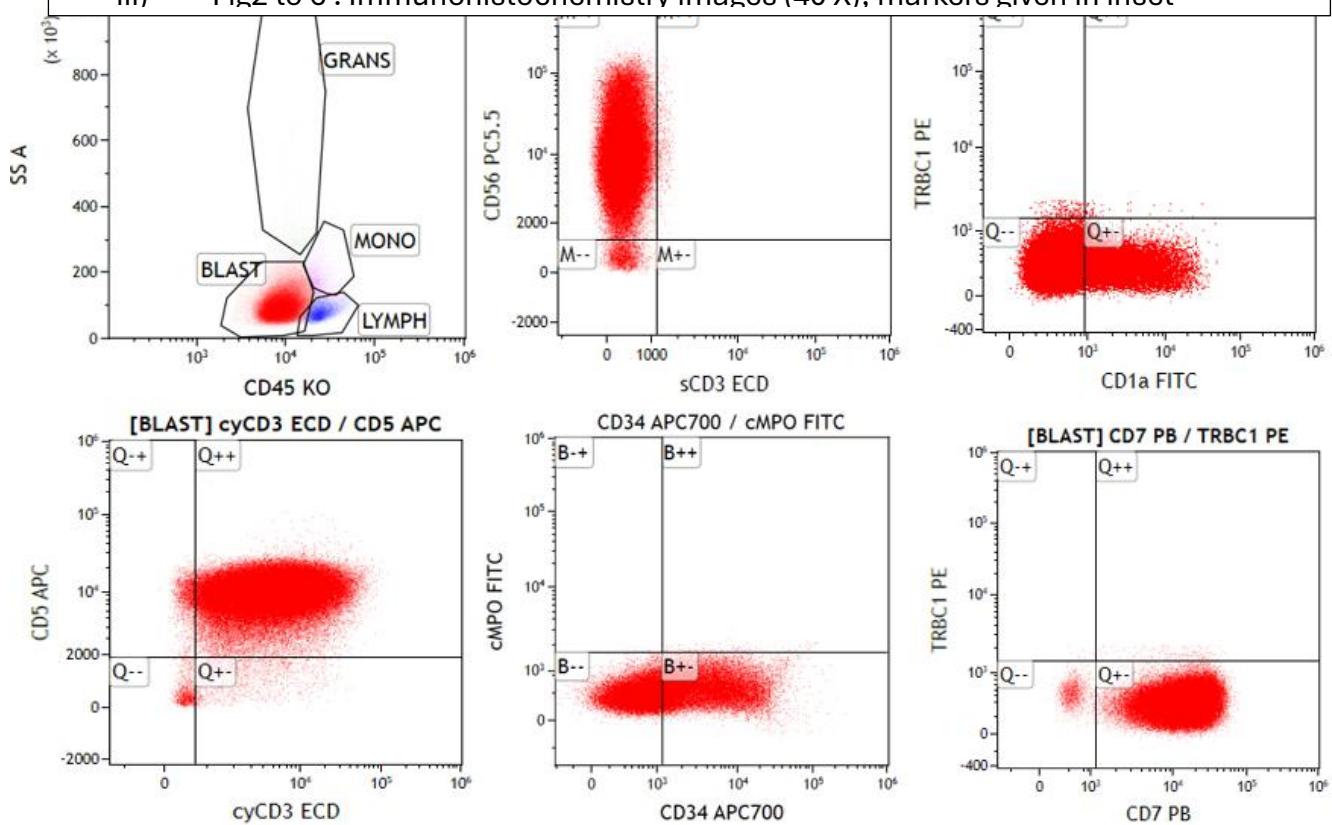
Case Presentation: We report the case of a 23-year-old male who was diagnosed with AML in July 2020. Bone marrow studies revealed 82% blasts, negative for MPO by cytochemistry. Flow cytometry confirmed the diagnosis of AML .The blasts were positive for myeloid lineage markers but negative B and T-lineage markers. AML multiplex basic panel did not identify any mutations. He received induction chemotherapy followed by maintenance. He achieved remission. HLA matched donor was not found so Allogenic hematopoietic stem cell transplant was not performed in this patient. Four years later, in July 2024, the patient presented with progressive bilateral cervical lymphadenopathy. Hematological parameters were within normal limits, and bone marrow examination revealed complete remission with 2% blasts. There was no hepatosplenomegaly. Lymph node biopsy revealed sheets of small to intermediate-sized atypical lymphoid cells. Immunohistochemistry showed diffuse positivity for CD3, CD5, CD7, with scattered weak positivity for Tdt, negative CD 4 and CD 8. Flow cytometry of the fine needle aspirate of the lymph node revealed 81% abnormal cells positive for CD7, CD5 along with immaturity markers like dim CD 45 , loss of sCD3 with retained cyCD3 , variable CD34 positivity and subset positivity for CD1a.TRBC1 confirmed the clonal nature of these cells and a diagnosis of T-ALL was made.

Conclusion: The occurrence of T-ALL following AML remission is rare and poses significant diagnostic challenges, particularly in distinguishing from T-cell lymphoproliferative disorders when Tdt is negative or weak. Extensive immunophenotypic work up including flow cytometry is mandatory to clinch the right diagnosis.

Image



ii) Figure 1: H&E image of Lymph node biopsy (40 X)
 iii) Fig2 to 6 : Immunohistochemistry images (40 X), markers given in inset



i) Flow plots of the lymph node aspirate ; markers depicted in the x and y axis

CP-09: Carryover of the ClearLLab 10C Panel on a DxFLEX Flow Cytometer: A Comprehensive Study

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Beckman Coulter Lifesciences Pvt LTD (BDC)

Introduction: Developing a diagnostic flow cytometer for IVD applications is vital for delivering rapid and accurate diagnostic information. However, the process of developing and validating these instruments is complex, influenced by factors such as sampling techniques, reagents, instrumentation, and data interpretation software. One crucial aspect of this validation is assessing carryover, which refers to the potential for residual sample contamination from previous analyses. Carryover can significantly impact accuracy and reliability of diagnostic results, making it essential to address in instrument qualification. This study aims to characterize and qualify the ClearLLab 10C panel, a 10-color dried tube designed for lymphoma and leukemia phenotyping on the DxFLEX flow cytometer. Carryover was evaluated as per CLSI (Clinical & Laboratory Standards Institute) H-26-A2 Guideline. Methods: Carryover performance was demonstrated on the ClearLLab 10C application, and the effectiveness of daily cleaning on DxFLEX flow cytometers was tested on DxFLEX QC fluorospheres and compensation beads. Specimen carryover was evaluated on three different lots of reagents using normal and clinical samples. The samples were diluted from (3) high-concentration samples (HTv) to arrive at (3) low-concentration samples (LTv, 1:10000 dilution) and acquired on three instruments. Carryover was calculated as: Results: The carryover performance on DxFLEX flow cytometers was demonstrated on the ClearLLab 10C application. Specimen and reagent carryover were well within the acceptance criteria of < 1% of the total WBC collected and, for DxFLEX daily QC Fluorosphere on specimens, were well within the acceptance criteria of < 0.5% of total beads collected, meeting the standard criteria. Conclusion: The comprehensive evaluation suggests that specimen and reagent carryover on three DxFLEX flow cytometers is within the specification of 1% of the total WBC, and therefore meet acceptance criteria.

Image

$$\% \text{ Carryover} = \frac{\text{LTv1} - \text{LTv3}}{\text{HTv3} - \text{LTv3}} \times 100$$

CP-10 : Sequential Cytokine Estimation using Flow Cytometry in Patients with B-cell neoplasms receiving anti-CD19-CAR-T therapy

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Tata Memorial Centre, Mumbai

Kasturba Medical College, Mangalore, Manipal Academy of Higher Education, Manipal

Background: Multi-parametric flow-cytometry(MFC) is widely used for identification of different diseases and immunological reactions as well as monitoring of chimeric-antigen-receptor(CAR)-T cells and MRD. CAR-T therapy has emerged as an advanced and efficient modality to treat patients with hematologic malignancies. Utilizing the functional property of immunomodulation by release-cytokines, new generation-CAR-T has been developed to target tumour cells. Cytokine-release-syndrome(CRS) occurs between 1st-14th day after CAR-T-infusion but role of MFC in CRS has not been established. ELISA is considered as gold standard method of cytokine estimation but is limited by higher cost, low throughput, need for higher sample-volume and is time-consuming. MFC-multiplex-bead-based-assays have overcome these hindrances.

Aims and Objectives: To study sequential cytokine estimation using MFC in patients with relapsed/refractory B-cell leukemia or lymphoma on CAR-T therapy. Methods: In this study, we established normal-range of cytokines and evaluated changes in their levels in cancer setting where patients were on anti-CD19-CAR-T therapy. Whole-blood was collected in K2-EDTA vacutainer tubes which were centrifuged at 1000xg for 10mins for plasma separation(50µL) done within 1-3 hours following blood collection. BD-CBA Flex set assays were procured for human IL-2/IL-6/IL-8/IL-10/IFN-γ/GM-CSF/MIP-1α and TNF-α. Beads were multiplexed for a single-tube-assay. Samples were acquired on BD-LSRFortessa™.

Results: Normal range of cytokines was evaluated in 45 healthy samples(Table.1). Cytokine profiling was performed in 233 plasma samples from 16 CAR-T patients [Mean age:20.6years;M:F ratio::2.2:1]. We noticed elevation in levels of these inflammatory cytokines in CAR-T patients particularly of IL-6,IL-8,IL-10,IFN-γ and MIP-1α. Our results showed a striking increase in the above 5 cytokines on day7 of CAR-T(Table.1). Additionally, we have used these assays to sequentially monitor the response to novel-therapeutic CAR-T therapy.

Conclusion: We have standardized the cytokine assay using flow cytometry and established their normal-reference values. We further demonstrated its applicability in detecting CRS and in monitoring immunological response reflecting anti-CD19-CAR-T-cell activity.

Sequential Cytokine Estimation using Flow Cytometry in Patients with B-cell neoplasms receiving anti-CD19-CAR-T therapy

Table 1: Median and range of cytokines evaluated in healthy and patients on CAR-T therapy

Sample	IL-2 (pg/mL)	IL-6 (pg/mL)	IL-8 (pg/mL)	IL-10 (pg/mL)	IFN-γ (pg/mL)	GM-CSF (pg/mL)	MIP-1α (pg/mL)	TNF-α (pg/mL)
LLOD (pg/mL)	0.00	2.43	4.08	1.17	1.00	0.00	1.02	1.00
Normal Mean(range)	10.14 (0- 135.94)	7.54 (0-87.73)	6.49 (0-14.91)	4.21 (0-59.35)	4.72 (0-22.4)	3.37 (0-51.55)	2.03 (0.5-12.03)	3.88 (0-77.08)
CAR-T Day-7	5.91 (0- 10.54)	513.97 (2.43- 3498.23)	646.60 (6.19- 200.39)	38.34 (3.92- 172.64)	40.56 (0-471.92)	0.96 (0-3.45)	7.94 (1.30- 13.66)	0.42 (0-1.43)
CAR-T Day-14	5.37 (0-9.51)	91.31 (1.98- 525.95)	28.82 (5.21- 79.36)	33.53 (4.46- 263.88)	12.95 (0-96.97)	0.70 (0-2.97)	6.72 (2.91-12.80)	0.69 (0-2.36)
p value	0.90	0.03*	0.20	0.67	0.97	0.20	0.52	0.46

CP-11: Utility of 16-color multiparametric flow cytometry in the diagnosis and staging of Classic Hodgkin lymphoma - experience in real-world practice

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Abstract

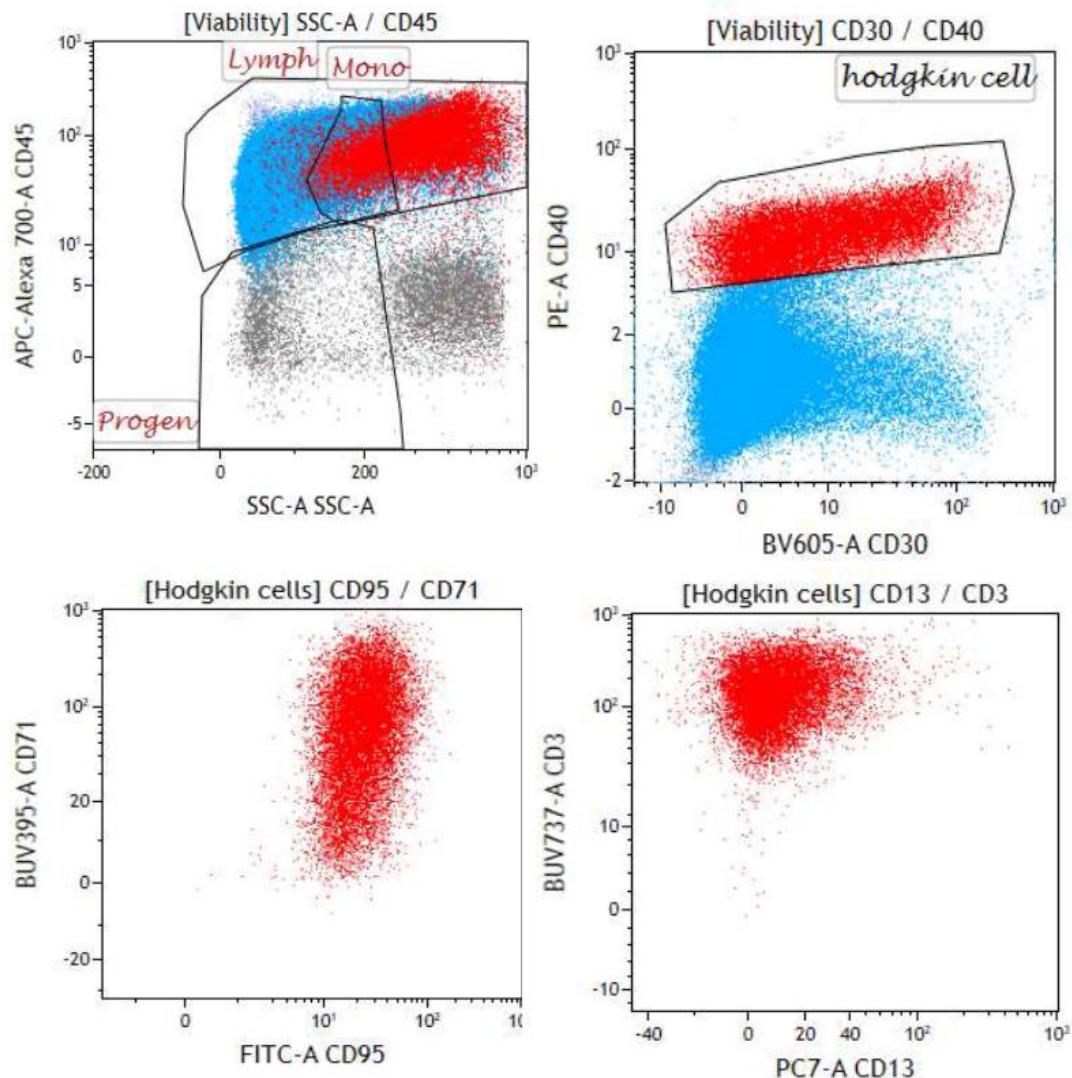
Background: Classic Hodgkin lymphomas (CHLs) are mainly diagnosed by histopathology and immunohistochemistry. Real-world data highlighting the role of flow cytometry (FCM) in the diagnosis of CHL is scarce. One of the main reasons is the scarcity of neoplastic Reed-Sternberg cells. This study aims to assess the utility of FCM in the diagnostic and staging of marrow samples of CHL in real-world practice.

Methods: This is a retrospective study of samples submitted for diagnosis/staging of CHL. Study included 106 samples from 103 patients (diagnostic=17;staging=89) comprising of FNACs (n=13), tissue biopsy (n=03), bone marrow aspirate(BM) (n=65) and body fluids (n=25). Samples were processed with bulk-lyse-stain wash protocol, acquired on BD LSRFortessa, and analyzed using Kaluza software. Cells were stained with single tube 16color antibody-panel that included CD3/CD11b/CD13/CD15/CD19/CD20/CD30/CD33/CD38/CD40/CD45/CD64/CD71/CD73/CD95 and CD271. The results were correlated with clinical, radiological and histopathological findings.

Results: Median age of patients was 34 years (range:3-81years) with M:F ratio of 2.8:1. CHL involvement was noted in 8 cases (7.3%) distributed as 3 FNACs, 2 BM, 2 plural fluids and one lymph node biopsy. CD30, CD40, CD71, and CD95 markers were used to diagnose CHL in these samples. Seven cases that did not show involvement by CHL on FCM were involved in histopathology. This could be attributed to sampling error from non-representative sites/hemodilution, etc. Additionally, on histopathology, FCM helped diagnose two suspected CHL cases that were earlier misdiagnosed as EBV-positive DLBCL. Two cases initially diagnosed as CHL on histopathology didn't show involvement by CHL on FCM and were reclassified as angioimmunoblastic T-cell lymphoma and reactive B-cell hyperplasia based on immunophenotype.

Conclusion: Immunophenotyping by flow cytometry is helpful in CHL diagnosis and staging in conjunction with histopathology. Flow cytometry for Hodgkin lymphoma can be a rapid method in guiding a more robust and integrated diagnosis of CHL.

Figure 1 showing Reed-Sternberg cells highlighted in red colour with high SSC and expressing moderate expression of CD40, CD71 and CD95; variable expression of CD30 and moderate CD3 (because of rimmimg by T cells).



CP-12: Relevance of <0.01% end of induction bone marrow measurable residual disease among pediatric patients with b-lineage acute lymphoblastic leukemia

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Introduction: End-of-induction measurable residual disease (EOI-MRD) of $\geq 0.01\%$ is an independent predictor for inferior outcomes among pediatric B-lineage acute lymphoblastic leukemia (B-ALL) patients. The prognostic relevance of EOI-MRD $< 0.01\%$ is largely unexplored.

Methodology: Clinico-laboratory characteristics and flowcytometric EOI-MRD among pediatric patients diagnosed with B-ALL between January 2018 to December 2022 were analyzed. Overall survival (OS) and relapse-free survival (RFS) among patients with EOI-MRD $< 0.01\%$ were compared with those having $\geq 0.01\%$ and undetectable EOI-MRD.

Results: Among 244 patients included, EOI-MRD was assessed among 223 patients, of which 81 (36%) had detectable EOI-MRD. Among these 81 patients, 18 (22.4%) had EOI-MRD $< 0.01\%$ (8% of all patients where EOI-MRD was assessed). There was no difference in the baseline hemogram, cytogenetics, and day 8 steroid response according to EOI-MRD status ($p > 0.05$). On median follow-up of 46 (95% CI: 43 to 49) months, EOI-MRD positive patients had a higher frequency of relapse than those without any detectable EOI-MRD (38% vs 18%, $p = 0.001$). Patients with EOI-MRD < 0.01 and $\geq 0.01\%$ had similar frequency of relapse (33% vs 40%, $p = 0.785$). Patients with EOI-MRD $< 0.01\%$ had a higher frequency of relapse than those with undetectable EOI-MRD (33% vs 18%, $p = 0.121$). Patients with undetectable EOI-MRD had superior 4-year OS (83% vs 60%, $p = 0.001$) and RFS (80% vs 61%, $p = 0.004$) than those with detectable EOI-MRD. There was no difference in OS (75% vs 55%, $p = 0.329$) and RFS (57% vs 57%, $p = 0.895$) between patients with EOI-MRD $< 0.01\%$ and $\geq 0.01\%$. Patients with EOI-MRD $< 0.01\%$ had inferior RFS (57% vs 77%, $p = 0.048$) but not OS (75% vs 82%, $p = 0.284$) than those patients with undetectable EOI-MRD.

Conclusion: Patients with EOI-MRD $< 0.01\%$ had similar relapse frequency, OS and RFS as those with EOI-MRD $\geq 0.01\%$. These patients also exhibited inferior RFS as compared to those with undetectable EOI-MRD. Hence, patients with $< 0.01\%$ EOI-MRD are also at higher relapse risk and warrant consideration for EOI treatment escalation.

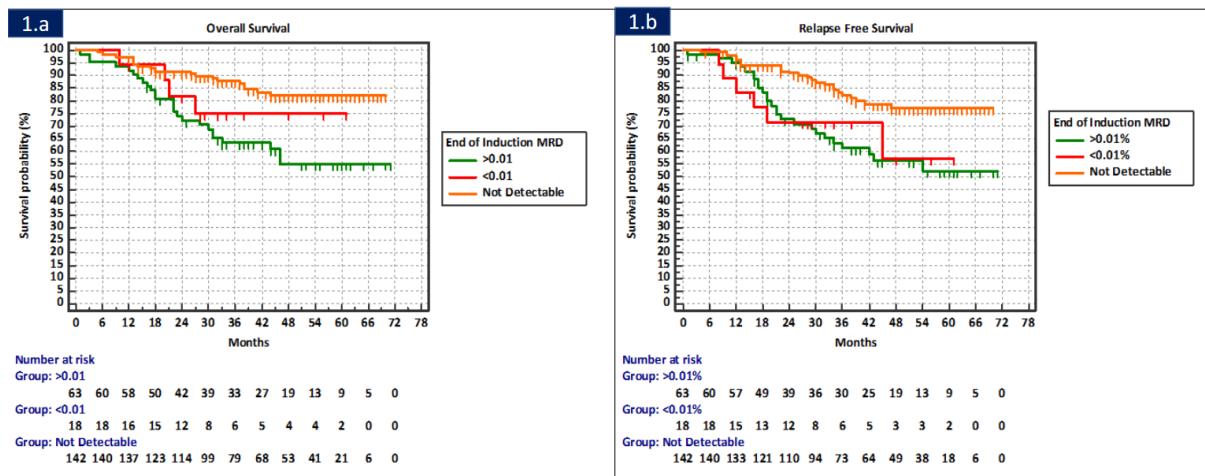


Figure 1. Overall survival (1.a) and relapse free survival (1.b) according to the level of end of induction measurable residual disease (EOI-MRD) among 223 consecutive pediatric B lineage acute lymphoblastic leukemia patients.

CP-13: Utility of slan expression and abnormal monocyte partitioning by flow cytometry in diagnosing chronic myelomonocytic leukemia

Dr. Pratishtha Madaan, Komal Tanna, Sitaram Ghogale, Nilesh Deshpande, Jagruti Patil, Karishma Girase, Gaurav Chatterjee, Sweta Rajpal, Nikhil V. Patkar, Papgudi G. Subramanian, Prashant R. Tembhare

Tata memorial center

Introduction: Chronic myelomonocytic leukemia(CMML) is a myeloid neoplasm exhibiting both myelodysplastic and myeloproliferative features. Diagnosis of CMML is often challenging due to overlap of its morphologic and genetic characteristics with those of other myeloid neoplasms and reactive monocytosis. Literature on role of flow cytometric immunophenotyping(FCI) in diagnosing CMML is limited to a few reports and real world data is still sparse.

Material and methods Bone marrow(BM) and peripheral blood(PB) samples were evaluated utilizing 16-color FCI panel that included antibodies against CD11b-PE594/CD13-PE/CD14-BV786/CD16-BV650/CD24-BV421/CD33-BV711/CD34-PER-CP/CD36-BV605/CD38-APC750/CD45-APC700/CD56-BUV395/CD64-BUV737/CD117-PC7/CD177-APC/HLA-DR-BV510/SLAN-FITC. Samples were processed using a bulk-lyse-stain-wash technique, acquired using BD LSRFortessa and analyzed with Kaluza software version-2.2.1. PB monocyte partitioning was performed using CD14 and CD16 (CD14+CD16- = classical monocytes(cMo),CD14+CD16+=intermediate monocytes(iMo), and CD14-CD16+=non-classical monocytes(ncMo). Proportion of ncMo was also studied using expression of "SLAN" protein. Final diagnosis of CMML vs non-CMML was done incorporating CBC, morphological, FCI, cytogenetic and molecular findings.

Results Study included 75 patients (40 CMML & 35 non-CMML). Of 40 CMML patients, 12/40(32.5%) were categorized into myelodysplastic-CMML & 25/40(67.5%) into myeloproliferative-CMML and on other hand, 8/40(20%) into CMML-1 and 32/40(80%) into CMML-2. BM morphology showed dysgranulopoiesis, dysmegakaryopoiesis and dyserythropoiesis in 70%,37.5%and 17.5%cases, respectively. PB-monocyte partitioning was used according to published cutoff of >94% for cMo and SLAN+ncMo <1.76% for CMML diagnosis. Table-1 showed the comparison between CMML and non-CMML group. In the CMML group,33/40(82.5%) also showed features of dysmonopoiesis based on maturation patterns of CD13vsCD15vshLA-DR and CD56 overexpression and 23/40(57.5%) showed dysgranulopoiesis based on CD13vsCD11bvsCD16 and CD56 overexpression. PB-monocyte partitioning had a sensitivity of 95.23% and specificity of 100%. Sensitivity of using >1.7% cutoff for SLAN+ non classical monocytes is 100% and specificity is 92.3%

Conclusion Peripheral blood monocyte partitioning using CD14 and CD16 along with expression of SLAN in non-classical monocytes is rapid, cost effective, highly specific and sensitive technique to diagnose CMML.

Table 1: Shows the comparison between CMMML and non-CMMML group.

Characteristics	CMMML(n=40) Median (range)	NON-CMMML(N=35) Median (range)
Age (years)	57(24-80)	48(26-75)
M:F(male to female ratio)	1.35:1	1.69:1
PB monocyte proportion (%)	10.3 (1.9-45.5)	8.74 (3.3-40.3)
Absolute monocyte count	1.67/ 10^9 /L (0.07-33.66 $\times 10^9$ /L)	1.10 (0.56-25.66 $\times 10^9$ /L)
FCI-PB monocytes proportion (%)	18.13 (3.3-42.5)	10.23 (2.5-25.6)
FCI-PB cMo proportion (%)	97.04 (94.4-99.55)	77.86 (22.85-99.32)
FCI-PB SLAN ncMO proportion (%)	0.9 (0.05-1.1)	2.7(1.5-7.5)

CP-14: Practical value of CD48 in T acute lymphoblastic leukemia MRD- using 13-color multi parametric Flow Cytometry.

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Advanced Centre for Treatment, Research and Education in Cancer, Tata Memorial Hospital, Navi Mumbai, India

INTRODUCTION

CD48 is an immunoglobulin superfamily receptor expressed on the surface of mature T-cells and NK-cells. It has been reported to be downregulated in T-ALL blasts and suggested to be useful marker in T-MRD. However, data on the utility of CD48 in T-ALL MRD monitoring is scarce and there is a need to evaluate its additional value in real world practice.

Methods

CD48 expression was studied in 217 samples including 27 diagnostic and 185 MRD from T-ALL patients. Samples were processed by bulk-lyse stain method, acquired using DxFLEX and analysed with Kaluza-software. Cells were stained with 13-color antibody panel that includes sCD3, cyCD3, CD4, CD5, CD7, and CD8, CD16/CD56, CD34, CD38, CD45, CD48, CD94 and CD161. Additional value of CD48 was studied and compared with conventionally standardized T-MRD approach.

Results

In 27 diagnostic samples, the CD48 expression was negative/downregulated in 24/27 cases (88.88%) with the median (range) proportion of CD48-negative blasts of 98.39% (62.23-100%).

Of 185 TMRD samples, 96 (51.89%) were MRD-positive using conventionally approach with median MRD of 0.23% (0.0009-87%). In MRD-positive samples, complete loss of CD48 expression was noted in 66/96(68.42%) and partial loss in 13/96(13.54%), thus supported the MRD detection in 81.96%. In 10 MRD-positive samples, the MRD was highly suspicious and loss of CD48 helped to confirm it. Notably, in 11 MRD-positive samples, the downregulation of CD48 expression had identified MRD in false negative samples with conventionally approach and hence it was the main marker to identify MRD. Of 90 MRD-neg samples, 18/90(20%) showed downregulation of CD48 in normal T/NK cells with median of CD48-negative cells 0.44% (0.0002-3.38%) which could result in false-positive MRD and was avoided using conventional approach.

Conclusions: CD48 has a definite additional value in TMRD monitoring. It should be interpreted cautiously with conventional T-MRD panel that essentially includes CD4, CD5, CD8, and CD38.

CP-15: Study of immunophenotypic signature of Anti-CD19 CART cells during initial expansion phase in paediatric BALL cases receiving CART therapy.

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Advanced Centre for Treatment, Research and Education in Cancer (ACTREC), TATA MEMORIAL CENTRE

Introduction: Chimeric antigen receptor (CAR)-T-cell therapy is considered as a major scientific breakthrough in cancer immunotherapy. Adoptive immunotherapy using CAR-T cells has achieved successful remissions in refractory B-cell leukemia and lymphomas. In order to estimate both success and side effects of CAR-T-cell therapies, monitoring of patient's immune profile including immunophenotypic signature of CAR-T cells is highly desirable. We analyzed immunophenotypic signature of anti-CD19-CAR-T cells in the initial expansion phase of paediatric relapsed/refractory B-ALL cases receiving CAR-T therapy.

Material and Methods: Immunophenotypic signature of anti-CD19-CAR-T-cells were studied in peripheral blood (n=60) and bone-marrow (n=12) samples from 12 B-ALL-patients using 14-color multi-parametric flow-cytometry (MFC) assay with CD3, CD4, CD8, CD19, CD20, CD45, CD62L, CD45RA, CD45RO, CD197 and Protein-L. We evaluated functional subsets of anti-CD19-CART-cells including naïve (Nv), effector memory (EM), central memory cells (CM) and terminal-effector-CD45RA+ (TERA) subsets in the product and patient's samples at different time-points i.e. Day-3, 5, 7, 14 and 28 during first month of therapy.

Results: Median (range) of CAR-T-cells in the product, Day3, Day5, Day7, Day14 and Day28 was 44.54(13.04-96.51), 15(1.11-29.02), 15.35(6.33-60.95), 40.81(6.8-84.48), 65.14(7.65-99.19) and 16.53(8.42-47.73)% respectively. Similarly, the median CD4:CD8 CAR-T cells was 0.70, 0.78, 0.93, 0.60 and 0.87. Fig.1a illustrated the dynamics of CAR-T cells, showing that their basal level (i.e. day 3-5) was minimum. However, the expansion started around 14th day and reduced at day 28. On further analysis of CAR-T functional subsets, we noted that at all time points, majority of CAR-T cells belonged to EM cells (median across all time points were 57.29, 53.94, 53.17, 79.39, 87.21 and 70.52) which was maximum during the peak expansion of CART cells. (Fig.1b).

Conclusion: Our study showed that the percentage of CAR-T cells was maximum around day 14-indicating that maximum expansion takes place in 2nd week of infusion. We also note that CAR-T cells are predominantly composed of functionally active EM cells during the peak expansion indicating its highest anti-tumor activity against CD19+ cells.

Fig.1a

CART cells expansion

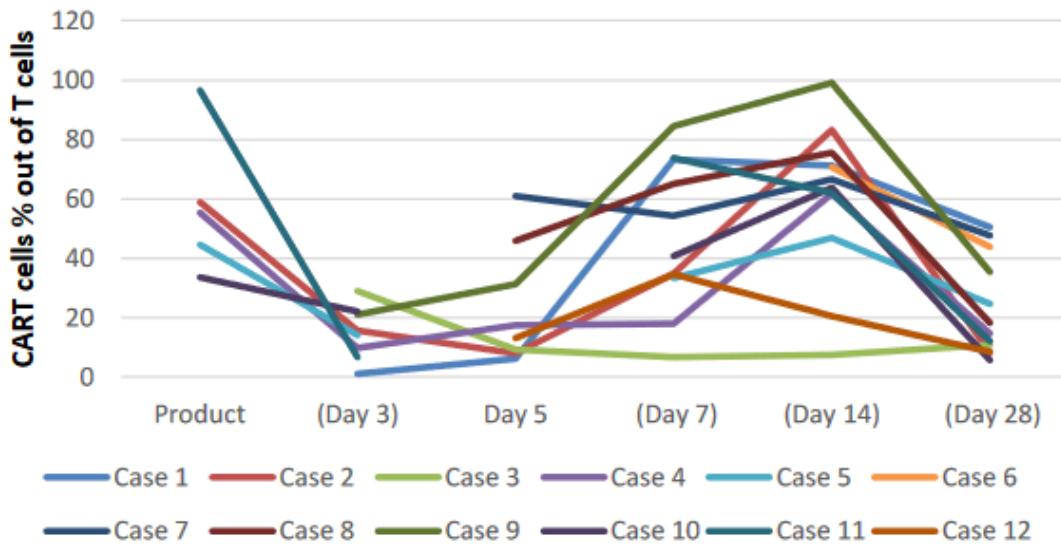
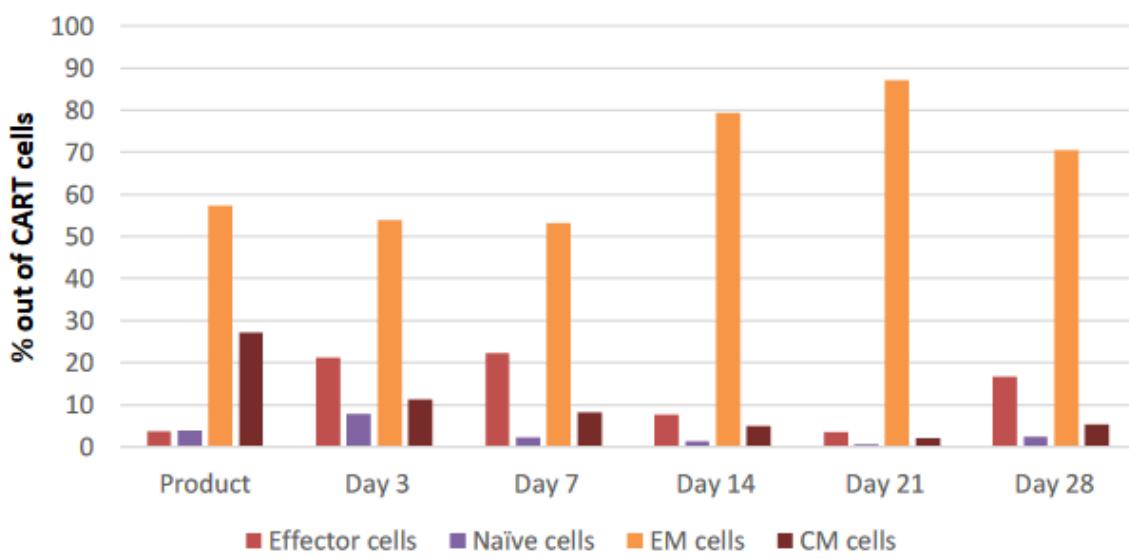


Fig.1b

CART functional subsets



CP-16: Standardization of the ClearLLab 10C Panel on DxFLEX Flow Cytometer: A Comprehensive Study

Vasanth Karunamoorthy, Naina Arora, Sneha Patel, Mehul Jivrajani, Anisha Jose, Prutha Patel, Kalyani Kotte, Aditya Jarare, Bharathi Ravi, Neha Girish.

Beckman Coulter Lifesciences, Bengaluru, India.

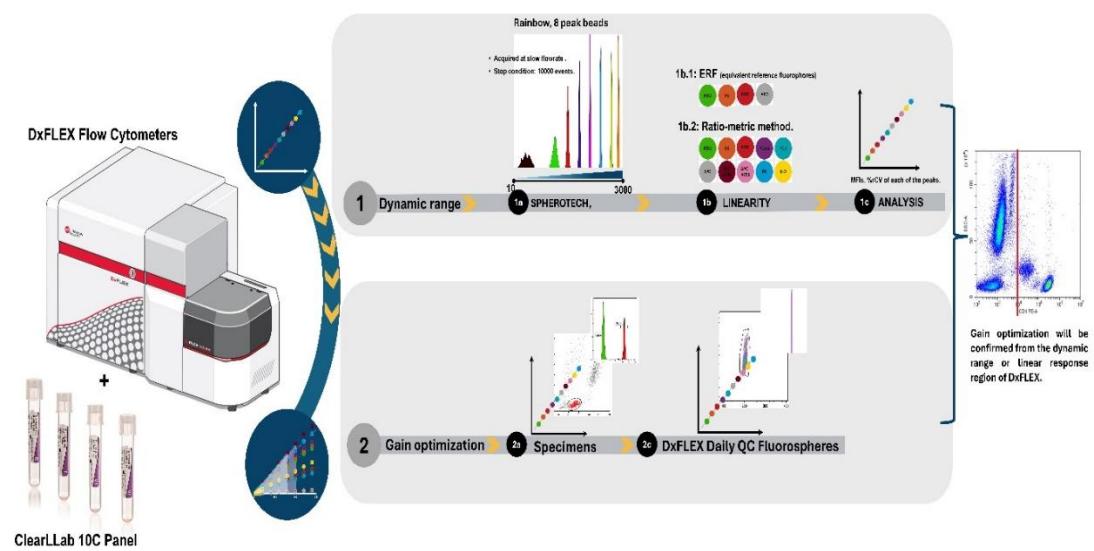
Background: The rapid growth of lymphoma and leukemia cases necessitates the development of in vitro diagnostic (IVD) products and precise testing methods for clinical use. Developing a diagnostic flow cytometer for IVD products is crucial for providing faster and more effective diagnostic information. However, the technical scope for developing IVD products/instruments remains variable due to factors such as sampling, reagents, instruments, and data interpretation software. To qualify an IVD product, characteristics and standardization processes are key. This study aims to characterize and qualify the ClearLLab 10C panel, an IVD product consisting of a 10-color dried tube for lymphoma and leukemia phenotyping, on the DxFLEX flow cytometer as per CLSI (Clinical & Laboratory Standards Institute) H-62 (Validation of assays performed by flow cytometry) guidelines.

Methods: The study involved optimizing the DxFLEX instrument for the ClearLLab 10C panel by testing for dynamic range and gain optimization, and carryover on four different instruments. Linearity was assessed using Spherotech rainbow calibration beads and demonstrated by ERF (equivalent reference fluorophores) and the ratio-metric method. The dynamic range was verified using biological specimens. The intensity of DxFLEX daily QC fluorospheres at different gains was measured and validated target values were within the scales of the DxFLEX instrument.

Results: The results confirmed the linearity of the DxFLEX instrument throughout the gain range, starting from 100 or 200 to 3000, based on the MFI, signal-to-noise ratio, and % rCV. Gain optimization was confirmed to be within the dynamic range and within the linear response region of DxFLEX daily QC fluorospheres.

Conclusion: The comprehensive evaluation suggests that the ClearLLab 10C application on the DxFLEX flow cytometer is suitable as aid in the differential diagnosis of leukemia and lymphoma.

Image



CP-17: Differential Expression of Surface Markers on Activated Platelets in Thyroid Cancer

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Introduction: Platelets (PLTs) are emerging as important non-invasive biomarkers in oncology. Tumor cells modulate platelet activation and aggregation through soluble agonists or direct interaction. Upon activation by agonists like ADP and thrombin, surface expression of CD62p (P-selectin) and CD63 increases, indicating α-granule release. Thrombin receptor-activating peptide (TRAP), acting via protease-activated receptors (PARs), strongly induces CD62p expression, marking a key step in platelet activation. This study examines platelet activation in thyroid cancer (Thy-Ca) patients and healthy donors (HDs), in relation to receptor modification status.

Method: Platelets were isolated from 10 ml of whole blood (n=13; 4 HDs, 9 Thy-Ca) via differential centrifugation and re-suspended in HEPES-Tyrode buffer. The population was split into active and inactive groups, with activation induced by ADP, TRAP, or thrombin for 20 min at room temperature. CD62p (FITC) and CD63 (BV711) expression were assessed using a BD FACS-Celesta flow cytometer (FC) and analysed with FlowJo software (v10.9). Gating based on FSC-A and SSC-A parameters was applied to isolate intact platelets by size and granularity.

Results: FC analysis revealed higher CD62p and CD63 expression in Thy-Ca PLTs at $32.4 \pm 0.79\%$ and $3052.11 \pm 0.51\%$, respectively, compared to HD-PLTs at $21.7 \pm 0.32\%$ and $2638.58 \pm 151.33\%$. ADP stimulation caused no significant change in HD-PLTs (CD62p: $24.70 \pm 0.32\%$; CD63: $2555 \pm 81.6\%$) but significantly increased expression ($P < 0.05$) in Thy-Ca PLTs (CD62p: $56.7 \pm 0.86\%$; CD63: $3114 \pm 49\%$). TRAP stimulation elevated CD62p and CD63 in HD-PLTs to $50.6 \pm 0.57\%$ and 2712 ± 18 , while Thy-Ca PLTs showed further increases to $81.54 \pm 1.06\%$ and $3226.81 \pm 48\%$ ($P < 0.05$).

Conclusion: Our preliminary findings demonstrate significantly higher CD62p and CD63 Expression in Thy-Ca platelets both at baseline and following stimulation with ADP and TRAP ($P < 0.05$). However, further studies with larger sample size are required to evaluate platelet activation as a potential marker for cancer therapy.

CP-18: B-ALL Residual Disease Detection: Multicolour Flowcytometry vs IgH-TCR MRD-A Head-to-Head in Today's Clinical Practice

Pratyush Mishra, Asish Rath, Sushant S. Vinarkar, Mayur Parihar, Jeevan Kumar, Niharendu Ghara, Vaskar Saha, Arijit Nag, Reena Nair, Deepak Kumar Mishra

TATA MEDICAL CENTER KOLKATA

Background - Minimal residual disease (MRD) is crucial for predicting outcomes in B-cell lymphoblastic leukemia (B-ALL). Although MRD negativity via standard flow cytometry (MFC) improves prognosis, relapse rates remain over 25%, possibly due to undetectable MRD. We evaluated a more sensitive MFC-MRD to detect low-level MRD in pediatric and adult B-ALL.

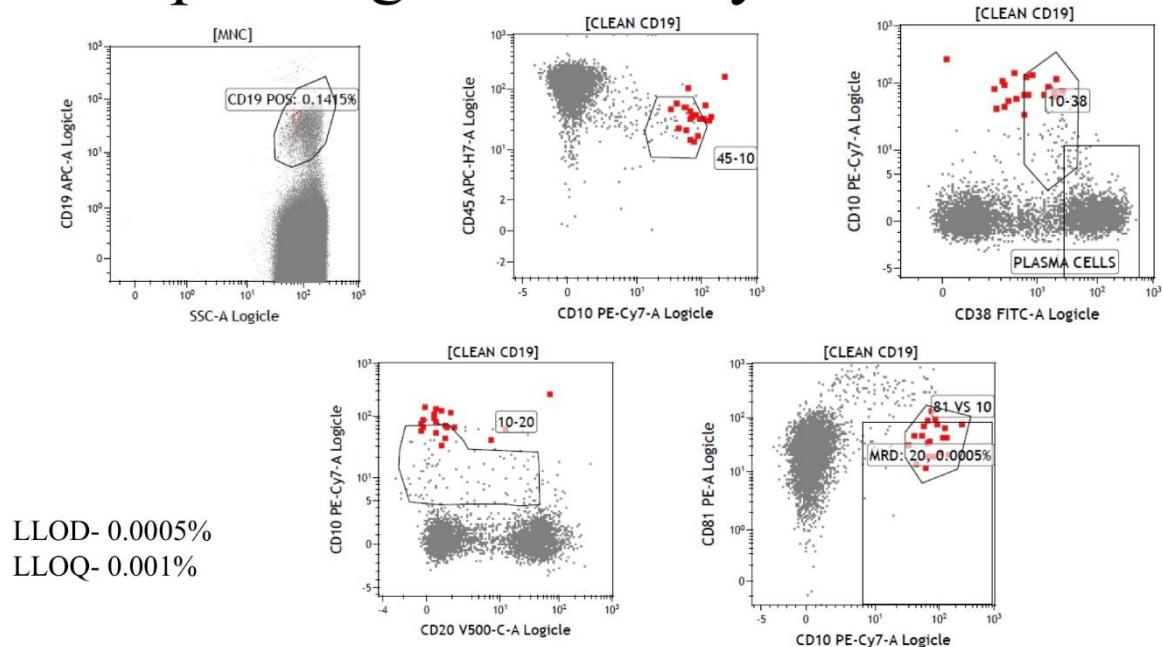
Methods - The study included all consecutive patients referred for B-ALL MRD from June-2022 to May-2024. A 11-color single tube B-ALL MRD was performed. Bulk lysis-stain-wash technique was adopted to acquire a minimum of 4 million viable event. All samples were acquired on BD FACSLyric 3-laser 12-color (BD Biosciences, San Jose) and analysed using Kaluza v2.2 software (Beckman Coulter Life science, California).

Results - 625 samples at different time point (post-induction- 300; 48%, post-consolidation-200; 32% and other time points- 125; 20%) were included in the study. Total MRD detected was 48% (MRD range 0.0005%-48%). Post-induction (PI) MRD detected was 36.6% (pediatrics-36%; 83/230, Adults-38.5%; 27/70). Of post-consolidation (PC) samples (n=200), 34 samples (17%) were MRD detected (pediatrics- 17.3%; 28/161, Adults-15.3%; 6/39). Rest of the samples from other time-points showed MRD detectability of 28.8% (36/125). Loss of CD81 was the most informative in our study helping in MRD identification in 79% cases followed by CD38 under expression (68%), over expression of CD10 (52%) and CD73 (55%). CD22 (14%) and CD20 (19%) were the least informative markers. 180 samples were consecutively analysed for both MFC and RQPCR (IgH-TCR), out of which 62 samples (48%) were MRD positive on MFC, 69 samples (53.4%) were MRD positive by IgH-TCR, 59 (45.7%) samples were MRD positive in both MFC and IgH-TCR. Considering IgH-TCR as gold standard, our concordance was 90% (62/69) with sensitivity of 85.5% and specificity of 95%.

Conclusion - High-sensitivity MFC-MRD and RQ-PCR detects minimal residual disease at lower levels, reducing false-negative results and providing a more accurate assessment of disease. This improved sensitivity supports personalized treatment and early relapse detection, allowing clinicians to adjust therapies proactively and enhance outcomes in both pediatric and adult B-ALL patients.

Image

Example- High Sensitivity



CP-19: Immunological predictors of chronicity in pediatric immune thrombocytopenia (ITP) patients: A flow cytometry approach.

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Umair Ahmed Bargir (ICMR-NIIH), Sangeeta Mudaliar (BJ Wadia hospital)

Dr Manisha Madkaikar(ICMR-NIIH)

Background: Immune thrombocytopenia (ITP) is an autoimmune disorder causing platelet destruction due to antiplatelet antibodies or T cell-mediated targeting. While many pediatric cases resolve within a year, some progress to chronic ITP. Identifying predictive markers for chronicity is essential to optimize treatment, minimize unnecessary interventions, and enhance patient outcomes.

Methodology: This study employed flow cytometry immunophenotyping to analyze 79 immune cell subsets in 93 patients. At presentation 41 acute ITP patients (going to complete remission) and 52 patients with chronic ITP were included in the study. The analysis focused on parameters such as absolute lymphocyte count (ALC), NK cells, and various T (naive, effector, TEMRA, memory) and B cell subsets (naive, class switch memory). Mann-Whitney U test and chi square test were performed to find differences in immune parameters between acute and chronic ITP patients followed by logistic regression to determine the best predictive markers for chronic ITP.

Results: The variable significant at 20% were included in the final analysis. Stepwise logistic regression identified three significant predictors of chronicity: increased class switch memory B cells (OR 1.101, 95% CI 1.006-1.206) (sensitivity=71.4% , specificity=61.5%), elevated TEMRA Tc-cells (OR 1.114, 95% CI 1.050-1.181) (sensitivity=75.5% , specificity=71.8%), and decreased ALC (OR 0.999, 95% CI 0.999-1) (sensitivity=74.4% , specificity=61.2%). Cut-off values for clinical utility were established: ALC below 3165 cells/ μ L (AUC: 0.681), class switch memory B cells above 8.12% (AUC: 0.652), and TEMRA Tc- cells above 11.76% (AUC: 0.744) to enhance clinical utility. Combining all 3 parameters improved the model AUC to 0.837 and sensitivity and specificity to 74.5% and 85.4% respectively.

Discussion: The findings suggest that increased class switch memory B cells and effector Tc cells, alongside lower ALC, are associated with chronic ITP. These markers reflect sustained immune activation and cytotoxic activity. The cut-off values provide practical thresholds for predicting chronicity, offering clinicians a tool for better risk stratification and management.

Conclusion: Integrating these immune markers into clinical practice can improve the prediction of chronic ITP in pediatric patients. This allows for more personalized treatment strategies, including earlier intervention with second-line therapies, potentially reducing unnecessary treatments in acute ITP and enhancing overall patient care and outcomes.

CP-20: Antigen expression patterns in Minimal/measurable Residual Disease of Acute Myeloid Leukemia : A Tertiary Cancer Care Centre Experience

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TATA MEDICAL CENTER

Introduction:- Acute myeloid leukemia (AML) is characterized by the clonal proliferation of undifferentiated myeloid precursors, known as blasts. The detection of residual disease (MRD) after therapy by flow cytometry is dependent on identifying immunophenotypic differences between leukemic cells and discrete stages of normal hematopoietic cells. Delineating over or under-expression of antigens, cross-lineage and asynchronous antigen expressions in leukemic cells helps in MRD analysis.

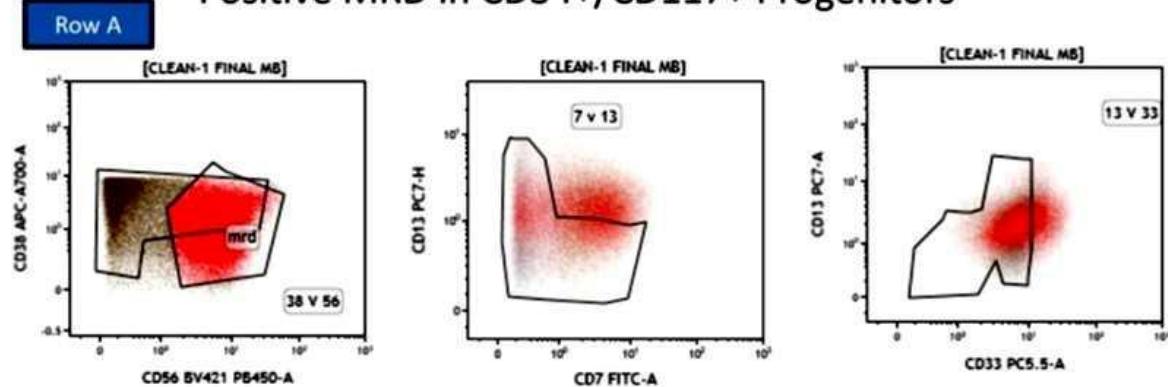
Aims and objectives: - To assess the different patterns of antigen expressed in MRD detection by Multicolor Flowcytometry (FCM) in patients with AML. Materials and methods:- First pull EDTA marrow was processed by Bulk-Lyse-wash protocol. Two million events acquired in most of the cases by using eleven-colour two tubes panel and analysed in Kaluza software (2.2) utilizing a hierarchical gating strategy with fixed gates to develop a LAIP-based Difference-from-Normal (DFN) approach.

Results: - Our study encompassed 66 adult patients ($>=18$ yrs, mean: 54 yrs) and 13 paediatric ($<=17$ yrs, mean: 11.5 years) in eight months duration. Aberrant phenotypes with or without expression of markers of immaturity were monitored in AML patients between and after completion of therapy. These patients received different treatment protocol and samples received for MRD at different time points. Sixty-nine cases were compared with LAIPs. 43% (n=34) cases are found MRD positive, ranging from 0.011 to 6.38 %. Aberrant CD56 and CD7 were found in 13.9% & 18.9% cases, CD19 found in 6.3% cases at diagnosis. In MRD analysis loss of expression identified in CD13(9.6%), CD34(3.2%), CD117(6.4%), CD56(9.6%), CD33(3.2%), CD38(6.4%), CD14(2.0%) and gain of expression in CD34(6.4%), CD117(6.4%), CD56(3.2%), CD7(9.6%), CD38(12.9%), CD64(6.4%) respectively.

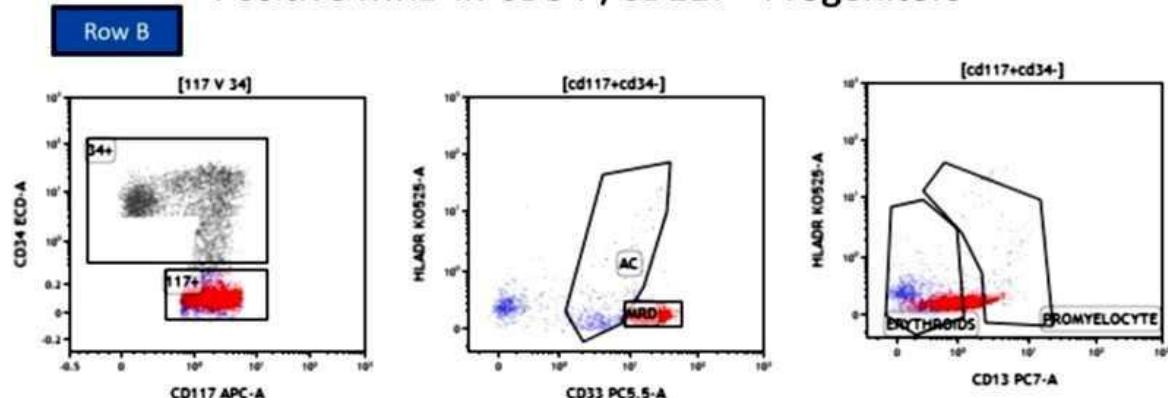
Conclusion:- Knowledge of normal patterns of early antigen expression with commitment to each of the major lineages is critical for MRD recognition. FCM represents highly sensitive technique for MRD detection & quantification and is applicable to almost all AML patients.

Image

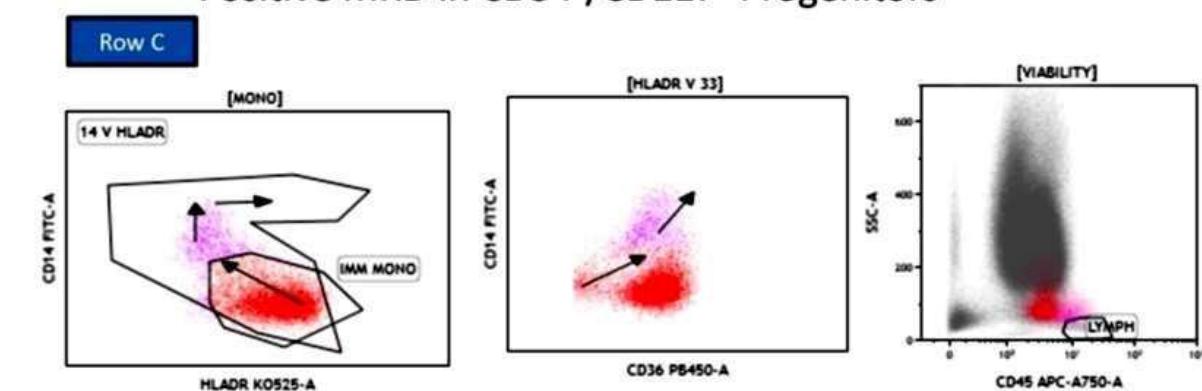
Positive MRD in CD34+/CD117+ Progenitors



Positive MRD in CD34-/CD117+ Progenitors



Positive MRD in CD34-/CD117- Progenitors



CP-21: The Role of Emerging Flow Cytometry Markers in Diagnosing and Monitoring Minimal Residual Disease in B-Lymphoblastic Leukemia/Lymphoma

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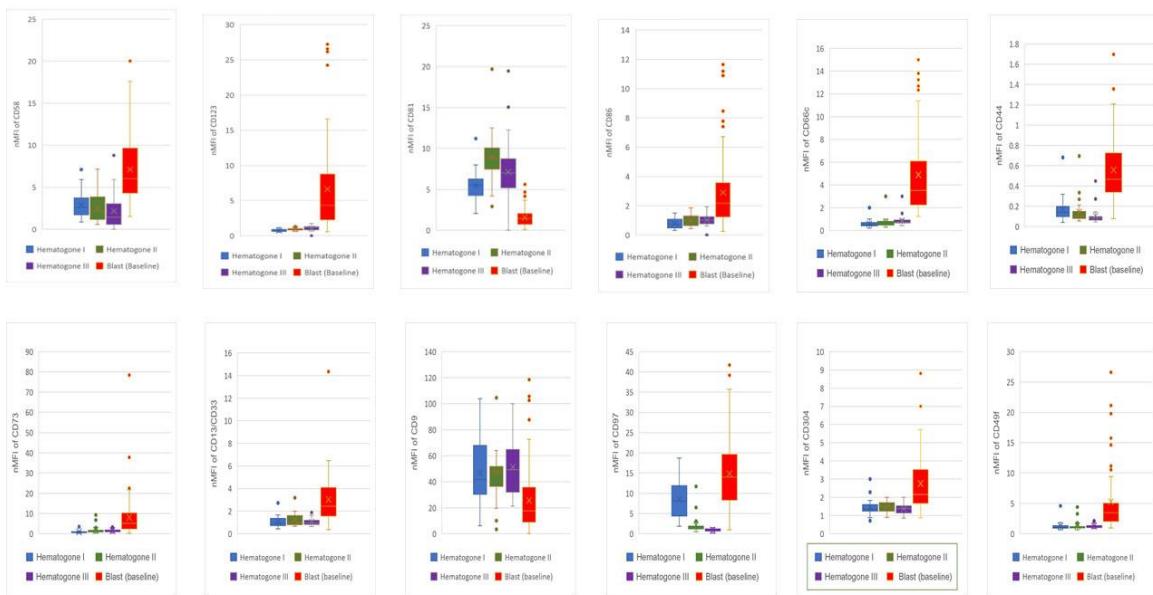
Post Graduate Institute of Medical Education and Research, Chandigarh.

Introduction: Measurable residual disease (MRD) is one of the profound prognostic indicators in B-acute lymphoblastic leukemia (B-ALL) and is commonly tested using multiparametric flowcytometry (MFC). We studied and compared 12 leukemia-associated immunophenotype markers namely CD81, CD86, CD123, CD58, CD9, CD13/CD33, CD44, CD97, CD66c, CD97, CD49f, and CD304 to identify the ones best suited for MFC-based B-ALL MRD detection.

Methods: The expression of above mentioned 12 markers were analysed in 103 baseline cases diagnosed in the Dept of Hematology, PGIMER, Chandigarh, India. Of these, 54 had post-therapy follow-up samples, of which 16 cases were MRD positive. Twenty-five bone marrow samples (not involved by B-ALL) were analysed to study the expression pattern in hematogones. Marker-expression was calculated as normalised mean fluorescence intensity (nMFI), which is a ratio of MFI of a specific marker in blasts to its MFI in mature B cells in the same sample.

Results and conclusion: 1. CD73, CD97, CD9 and CD86 showed aberrant expression in more than 85% of cases. 2. In MRD positive cases, CD81, CD13/CD33, CD73 and CD9 were retained as LAIP in 100% cases; CD58, CD97 and CD86 were retained in 93.7% cases; and CD49f was retained in 87.5% cases. 3. Of the cases showing overexpression of CD49f at follow-up, 45% showed negative CD49f at baseline, thus proving to be useful for MRD detection. 4. Of the 12 markers, CD73, CD97 and CD86 were found to be best suited to detect MRD in B-ALL due to their high frequency of expression and post-therapy retention.

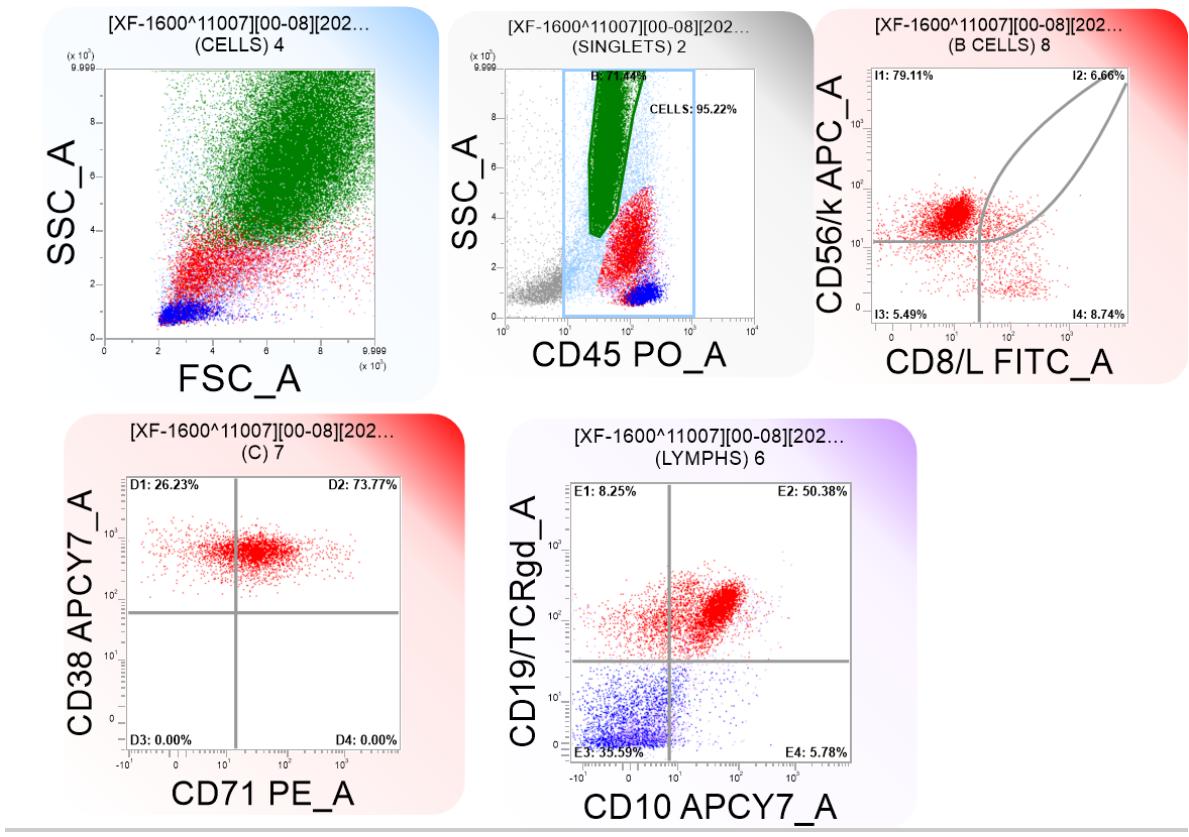
Image



CP22: Burkitt lymphoma with variant translocation - a case report.

**Krishna Dev D, Vishnu Vardhan K, Suresh Kotrakona, Wanve Balasaheb Ajinath,
Anuradha Sekaran**
AIG Hospitals, Gachibowli, Hyderabad

Burkitt lymphoma (BL) is a mature aggressive B-cell neoplasm composed of monomorphic, medium sized cells with basophilic cytoplasm, multiple small nucleoli, a GCB-phenotype, a high proliferation index and Ig::MYC rearrangement. A 40 year old male presented to our hospital with complaints of fever on and off, jaundice, vomitings, black stools since 2 months. Elsewhere diagnosed as NHL of stomach with periampullary growth. He was diagnosed as a case of spontaneous tumor lysis syndrome. Peripheral blood showed leucocytosis (WBC-30440/cumm) with few atypical cells. Bone marrow aspiration showed 25% abnormal lymphoid cells and trephine biopsy showed sheets, nodules of atypical lymphoid cells. Flow cytometry showed abnormal population (53%) with increased FSC, CD19+ve, CD20+ve, CD38 (strong+ve), CD10 (strong+ve), CD71+ve and CD43-ve, diagnosis of High grade B NHL with DDs of DLBCL vs Burkitt lymphoma was given. Karyotyping showed complex karyotype with balanced translocation between the long arms of chromosome 8 and 22, involving the regions 8q24 and 22q11 and trisomy 7,12, and additional X (49,XY,+X,+7,t(8;22)(q24;q11),+12[20]). The t(8;14)(q24.1;q32) translocation and its variants, t(2;8) (p12;q24.1) and t(8;22)(q24.1;q11.2) involve the MYC gene on chromosome 8 and are associated with B NHL, typically Burkitt lymphoma (BL). The variant translocations are less common and involve the IGK gene on chromosome 2p12 and the IGL gene on chromosome 22q11.2. Activation of MYC occurs on the derivative 14 in the t(8;14) and on the derivative 8 in the variant translocations. Additional translocations seen in 60–70% of cases, structural abnormalities of chromosomes 1q and 13q, trisomies 7 and 12, and losses of 6q, 13q and 17p. BL is an aggressive disease that has a poor prognosis; it may also be associated with immunodeficiency. Multi-parametric Flow Cytometry (MFC) is an indispensable and reliable diagnostic tool for the early detection of aggressive haematolymphoid neoplasms for prompt treatment.



CP-23: Chronic Myeloid Leukemia in Atypical Blast Phase: The unusual immunophenotypes

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Introduction: Chronic myeloid leukemia (CML) is a MPN driven by the BCR::ABL1 fusion or Philadelphia chromosome. Only 2.2% of CML cases may present in BP, which show ≥20% of blasts in the bone marrow or peripheral blood. Over 70% of the cases in blast phase have an immunophenotype(IPT) of myeloblasts, while 20-30% are lymphoblasts. Rarely they are monoblasts, megakaryoblasts or mixed phenotypical blasts. We report 3 cases of CML in BP with unusual IPT.

Case 1: 21 yrs old female presented with fever, weight loss and abdominal lump for 1 month. On examination, she had pallor, hepatosplenomegaly. The PBS showed anemia, leukocytosis with 85% large blasts having basophilic cytoplasm with cytoplasmic blebbing(A). Bone marrow biopsy showed near complete replacement by blasts, and blasts were CD41 and CD61 positive(B). Cytogenetic analysis showed t(9;22). IPT with clinical features were suggestive of BP in CML with Megakaryoblastic phenotype(C).

Case 2: 40 yrs old male known case of CML in CP on therapy, presented with low grade fever and increased blood counts. Cytogenetic analysis showed t(9;22) the time of diagnosis. PBS showed 63% blast, a diagnosis of CML BP was made(D). Compound mutation of ABL1 gene in exon 4 and exon 8 was found. IPT revealed blasts with Ambiguous Lineage favoring CML in BP with Undifferentiated phenotype(E,F).

Case 3: 39 yrs old male known case of CML in CP since 12 yrs, presented with fever, headache and whitish discharge from left ear. The PBS showed leukocytosis with 95% small to intermediate and few large blasts(G). A diagnosis of CML in BP was made. IPT was suggestive of B-Monocytic Mixed Phenotype blasts. Current status of the cases: 2 of the above listed patients have succumbed to the disease. One patient underwent Hematopoietic stem cell transplant (case1) and is currently doing well. Conclusion: CML in BP may show various phenotypes and are difficult to differentiate from Acute Leukemia when patients first present in BP. IPT plays a vital role in diagnosing such cases, as the incidence is rare and prognosis is poorer as compared to usual phenotypes that are found in CML BP.

CP-24: Use of phosphoSTAT1 as an alternative to interferon-stimulated gene expression to evaluate type 1 interferonopathies

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BACKGROUND

Interferonopathies are autoinflammatory and autoimmune disorders characterized by excessive production or signaling of type I interferons. Interferon-stimulated gene expression (ISG) is often used to diagnose and monitor disease activity in interferonopathies. Here, we assessed STAT1 phosphorylation by flow cytometry as an alternative technology to evaluate interferon signaling in monogenic interferonopathies.

METHOD

5 patients with genetically diagnosed interferonopathies were enrolled in this study. ISG expression and phospho-STAT1 levels were measured concurrently. Whole blood collected in heparin- anticoagulated tubes was used to measure STAT1 phosphorylation at baseline and in response to stimulation by IFN- α (type 1 interferon) and IFN- γ (type 2 interferon). Blood was collected in Tempus

tubes to assess ISG score and STAT1 gene expression to preserve gene expression. Relative mRNA expression was measured by qPCR using SYBR green. ISG score was calculated as the median fold change in the normalized expression of 7 interferon-response genes (IFIT1, IFI27, MX1, SIGLEC1, RSAD2, ISG15, IFI44L) compared to healthy controls. Cut-off was set at 2.15 calculated as median+2SD from a pool of healthy controls.

RESULTS

ISG was elevated in all patients, irrespective of disease activity and ongoing medications. ISG in patients ranged from (7.80-85.11). Additionally, STAT1 was consistently overexpressed in patients, with an average 2.5-fold increase compared to controls. Despite a positive ISG score and increased expression of STAT1, none of the patients tested had basal phosphorylation of STAT1. However, upon stimulation with IFN- α and IFN- γ , the median MFI of STAT1 in patients was 15556 and 17903, respectively, compared to same-day controls, with median MFI of 7851 and 7111.

CONCLUSION

Phospho-STAT1 by flow cytometry may serve as a sensitive marker for interferon signaling. It may serve as an additional functional tool to validate variants of uncertain significance found in patients with monogenic disorders

CP-25: DNA Ploidy Analysis Using FxCycle™ Violet (FCV) Based Flow Cytometry in B-Acute Lymphoblastic Leukemia

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All India Institute Of Medical Sciences, New Delhi

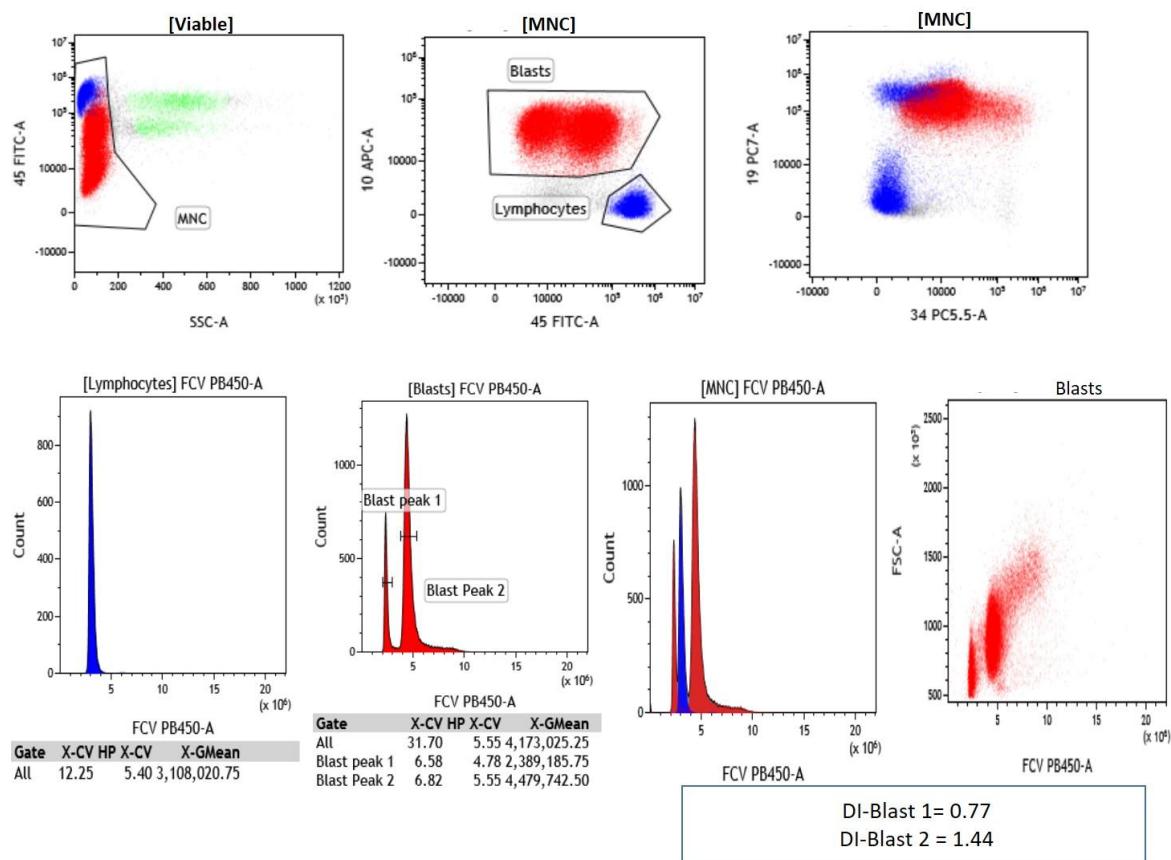
Background: DNA ploidy analysis is a vital tool for diagnosis and prognosis of hematological malignancies, including B acute lymphoblastic leukemia (B-ALL). It plays an essential role in guiding therapeutic decisions. FxCycleTM Violet (FCV) based flow cytometric (FCM) DNA ploidy analysis offers a powerful, rapid, and straightforward approach for analyzing DNA content in leukemic cells across a broad spectrum of hematological malignancies.

Materials and Methods: In this prospective study, we analyzed bone marrow (n=53) and peripheral blood (n=50) samples of consecutive new cases of B-ALL from May 2024 to August 2024 using FCV-based flow cytometry.

Results: DNA ploidy was assessed in 103 new B-ALL cases with a median age of 9 years (2 months–54 years) and a male-to-female ratio of 1.2:1. The overall DNA index (DI) ranged from 0.77 to 1.5. Diploidy (DI 0.96–1.05) was observed in 60 cases (58.3%), while aneuploidy was present in 43 cases (41.7%). Low-hyperdiploidy (DI 1.06–1.5) was found in 19 cases (18.4%), and high-hyperdiploidy (DI 1.16–1.39) in 21 cases (20.4%). Hypodiploidy was rare, with only three case (2.9%): one with exhibiting high-hypodiploidy (DI 0.89–0.95) and two cases with low-hypoploid (DI 0.70-0.88). These two cases showed endoreduplication, characterized by two distinct peaks on FCM-ploidy analysis: a hypodiploid peak and a hyperdiploid peak with nearly double the DI.

Conclusion: This study highlights the potential of FxCycle™ Violet in improving ploidy analysis workflows in clinical settings. Its ease of use, high precision, and ability to preserve cell viability make it a valuable tool in the ongoing efforts to optimize diagnostics and personalized treatment strategies in B-ALL. It efficiently detects aneuploidy in all B-ALL cases, even in peripheral blood or hemodiluted bone marrow samples with minimal blast populations or cytogenetic culture failure. Additionally, it enables the distinction between masked hypodiploidy and true hyperdiploidy.

Image



CP-26: Adult T cell leukemia/lymphoma - a case report.

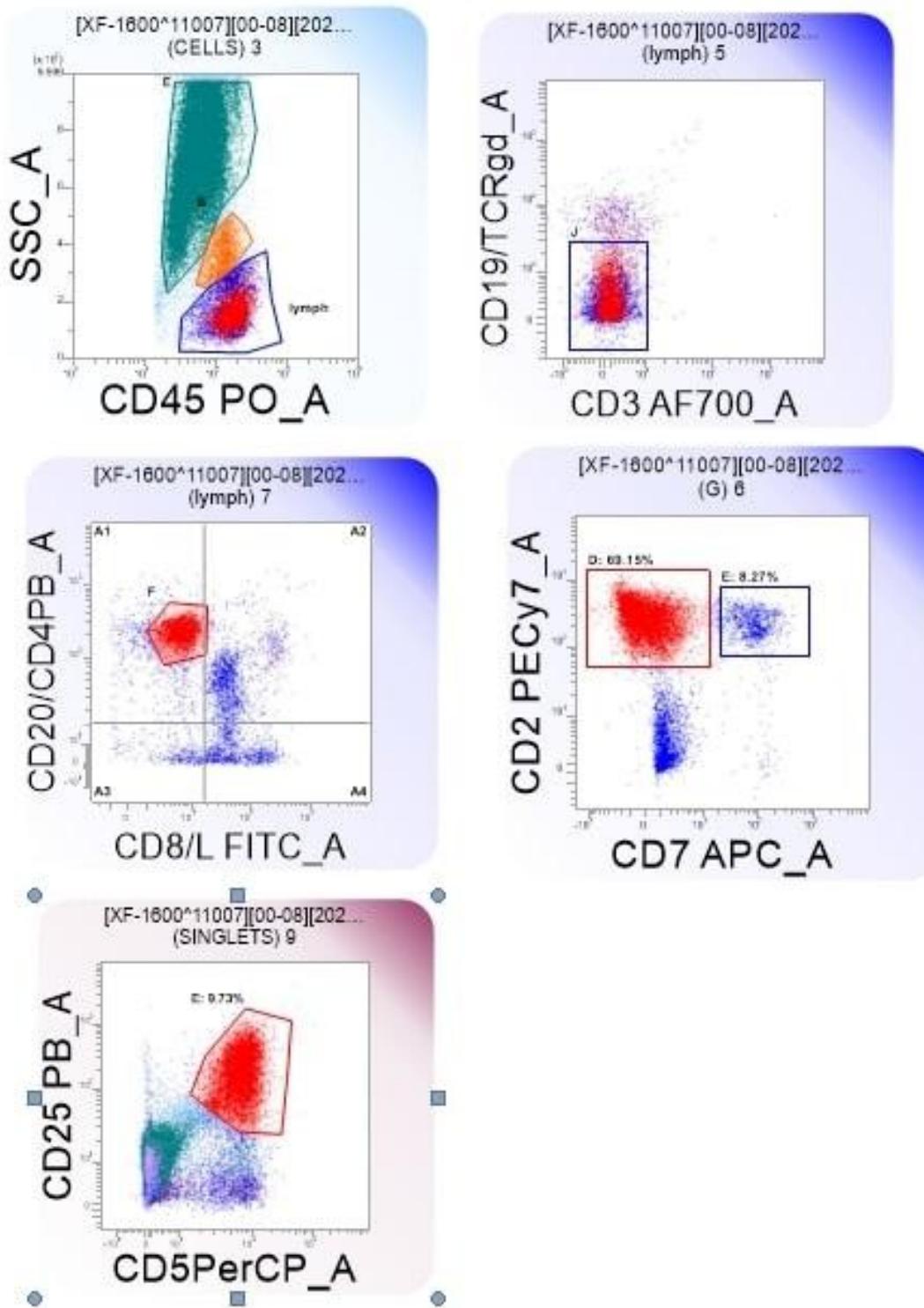
Dr Krishna Dev D, Dr Vishnu Vardhan K, Dr Safia Sultana, Mr Suresh Kotrakona, Dr Sohini Chattopadhyay, Dr Anuradha Sekaran
AIG Hospitals, Gachibowli, Hyderabad

Background and Introduction : Adult T cell leukemia/lymphoma (ATLL), one of the most aggressive among the peripheral T cell lymphomas, is a tumour of neoplastic lymphoid cell proliferation with mature T-cell phenotype with CD4+ T cells, caused by the oncogenic retrovirus, human T-cell leukemia virus type 1 (HTLV-1). We present a case report of a patient with ATL presented to our hospital.

Methods and Results: Our case was a 65 year old male presented with history of progressive increasing yellowish discoloration of eyes, skin for 8 to 10 days and hypercalcemia. History of loss of appetite and generalised weakness for 1 week. He also complained of whitish patches all over the body along with a reddish rash with skin thickening over the face and limbs. Blood investigations showed a total count of 28400/ μ l, Hb of 13 g/dl with LDH 663U/L. Peripheral smear showed left shift and atypical lymphoid cells- medium-sized to large cells, with irregular nuclei (30%). Bone marrow aspiration showed 26% atypical lymphoid cells and flow cytometric analysis showed abnormal population (15%) CD3 negative, CD4 positive population at lymphocyte gate, positive for CD2+, CD5+, CD25+, TCR ab+, CD8-ve and CD7-ve. HTLV1 antibodies was strongly reactive and the diagnosis was confirmed as ATL- leukemic type. CSF analysis: showed no evidence of disease. Ascitic fluid- showed atypical lymphoid cells. Cytogenetics revealed normal karyotype. MRI – showed features of acute hepatitis, disease infiltration was considered as a possibility and he was treated with Inj Cyclophosphamide with Inj Dexa. Post chemotherapy – bilirubin showed gradual improvement. Treatment Plan was 6 cycles of CHOP/ GCD – followed by AZT/ IFN maintenance, however patient wanted to continue treatment elsewhere.

Conclusion: Because of the extreme clinicopathologic heterogeneity, accurate identification requires combination of clinical features, morphology and immunophenotypic findings along with positive HTLV1 serology.

Image



CP-27: Flow cytometric immunophenotypic signatures in Myelodysplastic Neoplasm (MDS) using 16-color multi-parametric Flow Cytometry and their correlation with underlying genetic abnormalities

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Introduction- MDS is a clonal disorder characterized by cytopenias and dysplasia in hematopoietic-cell lineages. Flow cytometry(FCM) and genetic testing are important for diagnosis and prognosis as it provides insight into dysplastic changes and clonal evolution.

Aims and objectives- 1. To identify distinct FCM marker profiles associated with different subtypes of MDS 2. To correlate immunophenotypic profile with underlying genetic abnormalities

Materials and methods- Ninety-three bone marrow aspirates from patients diagnosed as MDS by morphology and FCM along with their genetic profile were evaluated(January2023-August2024). Samples were processed by bulk-lyse-stain method, acquired using LSRFortessaTM and analysed with Kaluza-software 2.2.1. These findings were correlated with genetic profile wherever available.

Results: Median age of patients was 61years(5-88y) with M:F(1.57:1). Morphology revealed tri-lineage dysplasia in 17.20%(n=16), bi-lineage in 35.48%(n=33), and uni-lineage dysplasia in 36.55%(n=34). Immunophenotypic features of MDS were noted in 100% of cases. Of the 93 cases, genetic studies were available for 92.47%(n=86). There were no genetic abnormalities in 33.72%(n=29) despite dysplastic features. Most frequent abnormality in myeloblast was down-regulation of CD38(n=52). Granulocytes mostly showed asynchronous maturation pattern with respect to CD13vsCD16vsCD11b(n=56). Monocytes most frequently displayed abnormal maturation patterns of HLA-DRvsCD14(n=23). Erythroid series showed most commonly CD71 down-regulation(n=47). Hematogones were reduced in 75.26% cases(n=70). Specific genetic abnormalities and its correlation with flow markers are summarized in Table 1. MDS with 5qdel(n=3) showed most commonly CD38 down-regulation along with aberrant CD56 overexpression in myeloblast(n=2) while MDS with bi-allelic TP53 inactivation(n=6) showed bright CD13 with aberrant CD7 overexpression in myeloblasts(n=4). MDS with SF3B1 mutation(n=3) showed dyserythropoiesis, mostly down-regulation of CD71(100%).

Conclusion- Flow cytometry is highly sensitive for diagnosing MDS especially when the genetic testing is negative. Immunophenotypic signatures, such as CD38/CD56 expression in MDS with 5qdel and CD13/CD7 expression in MDS with bi-allelic TP53 inactivation, can potentially predict underlying genetic abnormalities.

Image

Table 1a- Flow cytometric immunophenotype showing dysplastic changes in different cell lineage

Myeloblasts	% (No.)	Granulocytes	% (No.)
Abnormal down-regulation of CD38	58.06% (n=54)	Abnormal granularity (low SSC)	38.70 % (n=36)
Abnormal expression of HLA-DR	44.08% (n=41)	Abnormal maturation pattern with respect to CD13vsCD11bvsCD16.	61.29% (n=57)
Expression of CD56	27.95% (n=26)	Down-regulation of CD177 in neutrophils	44.08% (n=41)
Expression of CD7	39.78% (n=37)		
<hr/>			
Erythroid	% (No.)	Monocytes	% (No.)
Abnormal under expression of CD71	52.69% (n=49)	Abnormal maturation pattern with respect to HLADRvsCD14	24.73% (n=23)
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B cell progenitors	% (No.)		
Decreased	75.26% (n=70)		

Table 1b- Flow cytometric immunophenotype and their correlation with genetic abnormalities

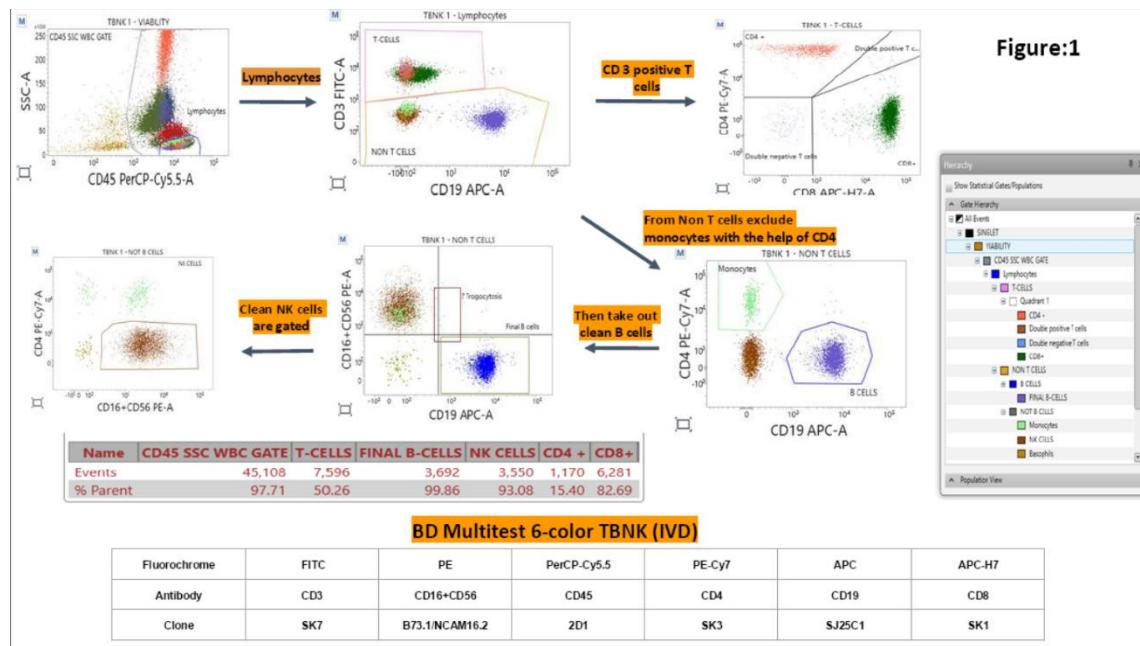
Genetic category	Myeloblast	Erythroid
MDS with low blast with 5q deletion (3 cases)	Abnormal down-regulation of CD38	Normal
	Abnormal overexpression of CD117	
	Aberrant overexpression of CD56	
MDS with low blast with SF3B1 mutation (3 cases)	Normal maturation pattern	Abnormal down-regulation of CD71
MDS with biallelic TP53 inactivation (6 cases)	Aberrant overexpression of CD7	Abnormal down-regulation of CD71
	Abnormal down-regulation of CD38	Increased CD105 positive and CD117 positive early erythroid precursors
	Abnormal overexpression of CD117 and CD36	

CP-28: Novel gating strategy using CD 4 as a monocyte marker for accurate enumeration T-cell , B-cell and NK-cell subsets.

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Neuberg Sehgal Path Lab

Lymphocyte (T-cell, B-cell, Natural Killer[NK]-cell) subset analysis have been widely accepted especially in the setting of immunodeficiency, immune dysregulation, immune reconstitution (post bone marrow transplant) and hematological malignancies. The widely practised methods to isolate and enumerate TBNK subsets by flowcytometry include combining CD45 with side scatter, followed by specific T, B, NK lineage markers to distinguish various lymphocytic populations. However, this strategy may be inadequate in patients with monocytosis; especially where there is an increase in non-classical monocytes and in patients with lymphopenia. As we know non-classical monocytes express both CD4 and CD16 and are negative for CD14. Hence most gating strategies end up including CD16 positive non-classical monocytes into NK cells. Recently, a number of studies have included CD14 as the gating marker to exclude monocytes. However, non-classical monocytes being CD14 negative are not effectively excluded from the target population. We hereby present a novel gating strategy where CD4 is used for excluding monocytes more accurately; without the need for any other additional monocytic marker. Our detailed antibody panel and gating strategy is illustrated in Figure 1. Post CD3 based T-cell gating, CD4+ve monocytes are excluded from non-T-cells. Additional gates are also made to exclude CD16/CD 56+ CD19+ trogocytotes which would otherwise be included along with B cells by conventional methods. In addition, we will share additional gating strategies during the final presentation, for estimating flow based accurate five-part WBC differential using the same panel. This approach provides an accurate absolute lymphocyte count; especially in lymphopenic patients where CBC machine-based differentials are unreliable. In conclusion, this novel gating strategy is an economical and more accurate method for estimating Lymphocyte subsets with the added bonus of getting a five-part WBC differential.



CP-29: Eye lid cleansing using medicated lid wipes reduced ocular surface inflammatory factors in healthy volunteers.

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Background: Muco-cutaneous junctions of the eye lid are pivotal in regulating ocular surface health. Pathological changes in lid margins contribute to ocular surface diseases with aberrant inflammatory response resulting in severe ocular discomfort and at times, blindness. Hence, lid margin health maintenance is essential in the prevention and management of ocular surface diseases.

Objective: To assess ocular surface inflammatory profile changes following use of medicated lid wipes in healthy volunteers.

Methodology: Study cohort included healthy volunteers (n=10; 20 eyes). They underwent lid margin cleansing process daily for 4 weeks using 2 different types of lid wipes. Tear fluid(TF) was collected using Schirmer's strips prior to lid cleansing(PW) and at 1 week(1W) and 4 weeks(4W) thereafter. TF was eluted from the Schirmer's strip and the levels of IL-2, IL-4, IL-5, IL-6, IL-9, IL-10, IL-13, IL-17A, IL-17F, IL-22, MMP2, MMP9, TIMP1, MMP9/TIMP1 ratio, IFNg, TNFa, b2-microglobulin, EPO, MPO, PDGF-AA and PDGF-BB were measured by bead-based multiplex ELISA using flow cytometry. Friedman test and 2-way ANOVA with multiple comparison test was performed.

Results: An overall reduction in majority of the TF inflammatory factors was observed following lid margin cleansing at 4W compared to PW. IL-4, TNFa & IFNy levels were significantly($P<0.05$) lower in 4W compared to PW. No significant difference was observed between the two lid wipes in reducing the TF inflammatory factors.

Conclusion: Medicated lid wipe-based lid margin cleansing reduced ocular surface inflammatory factor levels. Hence, lid hygiene procedures have the potential to reduce ocular surface inflammation and associated diseases including blindness.

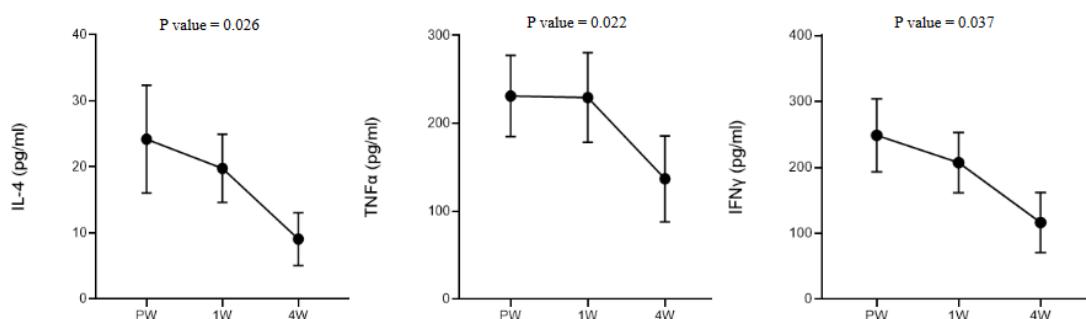


Fig1: Inflammatory factors IL-4, TNF α & IFNy levels in Tear fluid. PW = prior lid cleansing, 1W= 1 week, 4W= 4 weeks

CP-30: Impact of Additional Cytogenetic Abnormalities on Measurable Residual Disease Outcome in Philadelphia Chromosome-Positive Acute Lymphoblastic Leukemia

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Background: Philadelphia chromosome-positivity (Ph+) in acute lymphoblastic leukemia (ALL) is a high-risk cytogenetic abnormality. The co-existing high-risk additional cytogenetic abnormalities (ACAs) are linked to poor outcomes. However, the impact of ACAs on measurable residual disease (MRD) status and their potential as early markers for poor outcomes is unknown. This study aimed to determine the effects of ACAs on MRD outcomes in newly diagnosed Ph+ B-ALL.

Objectives: 1. Determine the frequency of Ph+ B-ALL 2. Characterize the additional cytogenetic abnormalities in Ph+ B-ALL 3. Correlate the additional cytogenetic abnormalities with MRD outcomes
Methodology: We retrospectively analyzed ALL patients seen at the Christian Medical College, Vellore, between 2014 and 2024 who underwent cytogenetic and flow cytometry-based analyses. Unsupervised cluster analysis was performed based on diagnostic flow cytometric data.

Results: In our cohort, 6.8% (148/2166) were de-novo Ph+ B-ALL cases. The median age was 28 years (range: 1-70 years) with male to female ratio of 1.5:1. Among these, 122 cases (82.4%) had Ph+ chromosomes with ACAs (ACAs+), while 26 cases (17.6%) had only Ph+ chromosomes (ACAs-). The ACAs+ group was further divided into high-risk ACAs+ (HR-ACAs+) (24 cases) and low-risk ACAs+ (LR-ACAs+) (17 cases). MRD data was available for 55 cases, showing MRD positivity in 14 (58.3%) HR-ACAs+, 10 (58.8%) LR-ACAs+, and 8 (57%) ACAs- cases, with no significant difference between groups. Based on CD markers, unsupervised cluster analysis of 19 diagnostic samples revealed two distinct clusters but no significant difference in ACAs between the clusters. However, there was a trend toward higher MRD positivity in clusters with more immature phenotypes.

Conclusion: Additional cytogenetics abnormalities in Ph+B-ALL did not significantly alter the MRD outcome. However, the present study cannot comment upon its use as a predictor for overall and relapse-free survival.

BASELINE CHARACTERISTICS OF DE-NOVO Ph+B-ALL

De-novo Ph+ B-ALL	Total (n=148)	No ACAs (n=26)	ACAs (n=122)
Median Age (yrs)	28	28	29
Age Range (yrs)	1-70	2_70	1_63
M:F ratio	1.46:1	2.3:1	1.3:1
Female	60	8	52
Male	88	18	70
Age Group			
Children(<15 Yrs)(%)	41 (27.7)	8 (30.8)	33 (27)
Adolescent(16-39 Yrs) (%)	67 (45.3)	12 (46.1)	55 (45.1)
Adult(>=40 Yrs) (%)	40 (27)	6 (23.1)	34 (27.9)
MRD Status	55	14 (25.4)	41 (74.6)
MRD+ve(%)	32 (58.2)	8 (57.1)	24 (58.5)
MRD-ve(%)	23 (41.8)	6 (42.9)	17 (41.5)
MRD+ve(Median) (Range)	0.02 (0.001-66.4)	0.015 (0.001-0.860)	0.032 (0.002-66.4)

CP-31: Exploring CD73 absence in B-ALL with KMT2A rearrangement: New insights into Immunophenotyping

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Introduction: B-Cell acute lymphoblastic leukemia (B-ALL) a hematological malignancy, is characterized by uncontrolled clonal expansion of B-lymphoid progenitors in bone marrow (BM) and other medullary sites. KMT2A on chromosome 11q23 encodes a histone methyltransferase that promotes transcription by opening chromatin. Breakpoints in KMT2A occur in about 10% of acute leukemia and are associated with various hematologic disorders. Notably, B-ALL with KMT2A rearrangements is rare and has a poor prognosis. In this scenario, we have studied CD73 negative expression in conjunction with KMT2A rearrangement in a small cohort.

Methods: We studied the expression of CD73 (BV605,Clone-AD2) in total 117 newly diagnosed Acute Lymphoblastic Leukemia, out of which 18 B-ALL cases at both diagnostic and post-treatment time point between duration of January-August 2024. FCI for diagnostic time-point was performed on 12-color BD-FACS Lyric and for the post-treatment time-point on 13-color Beckman Coulter Dx-Flex instrument. The data-analysis was carried out on BD-FACS suite and Kaluza software. Cytogenetic abnormalities were detected by standard fluorescence in-situ hybridization (FISH).

Results: We analyzed the expression of CD73 in a total of 18 B-ALL cases. Of the 18 cases assessed, 7 (38.88%) showed negative CD10 expression, and 3(42.85%) were CD73 negative. FCI of the B-ALL cases showed expression of CD19, CD22, cytoCD79a positive, with CD10 negative and aberrant expression of CD15. The median age of the patients was 46 years (range, 9-50 years) and the median white blood cell count at diagnosis time-point was $255.5 \times 10^9/L$ (range, $285.8-433.7 \times 10^9/L$) with hyperleukocytosis. Out of 3 cases, MRD of only 1 case was studied. The patient was not in remission and subsequently died. On further cytogenetic analysis, these CD73-negative cases were positive for KMT2A rearrangement.

Conclusion: Our study aims to emphasize that CD73 negativity in B-ALL appears to be associated with KMT2A rearrangement. In many B-ALL cases of CD73 positive expression along with other LAIPs known, our study focuses on the negative expression association with KMT2A rearrangement. Additional studies are required on a larger cohort. Absence of CD73 expression by independent immunophenotyping in B-ALL is in syndication with KMT2-MLL rearrangement is cognate to poor prognosis.

Characteristic	KMT2A (n=3)	Non-KMT2A (n=15)
Age	46 (9-50)	26(2-69)
Gender	Female-3	M:F (11:6)
WBC ($\times 10^9/L$)	255.5 (285.8-433.7)	37 (4.6-423.1)

CP-32: Immunophenotypic and morphologic differences between HLADR negative and positive acute myeloid leukemia

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Background: Under-expression of HLA-DR is a recognized characteristic of both normal and leukemic promyelocytes in Acute Promyelocytic Leukemia (APL). However, this immunophenotype is not specific for APL. These HLA-DR negative non-APL cases are further referred to as HLA-DR negative AML in contrast to APL and HLA-DR positive AML. They have morphologic features similar to hypogranular APL along with abnormal coagulation and fibrinolysis. There is a paucity of literature that describes the relationship of the morphology and immunophenotypic features of HLA-DR negative AML compared to HLA DR positive AML. We aimed to study the differentiating features of HLADR negative versus HLADR positive AML. APL versus non APL AML.

Methods: A retrospective observational study was performed after approval from institutional ethics committee (IEC No.-64/21). Cases of newly diagnosed AML over past 4 years were reviewed. Acute leukemia of ambiguous lineage and patients <14 years of age were excluded. Clinical features, complete blood counts, peripheral smear and bone marrow morphology, immunophenotype, cytogenetics and molecular testing reports were collected. Appropriate statistical tests were applied. A p value of <0.05 was considered statistically significant.

Results: A total of 63 cases were included in the study. HLADR was negative in 31 (49.2%) cases. Among these cases, 21 were diagnosed as APL, and 10 cases were non APL- AML. Expression of CD34 and CD7 was significantly different between the groups. Absence of CD34 and strong MPO strongly correlated with morphologic diagnosis of APL and PML::RARA translocation. NPM1 mutation was seen in 3/63 (4.7%) cases and had strong CD34 expression. FLT3/TKD mutation was noted in 4/63 (6.3%) and FLT3/ITD was seen in 3/63 (4.7%).

Conclusion: This study highlights the significance of a comprehensive diagnostic approach in distinguishing APL from HLA-DR negative AML. This integrated strategy holds promise in effectively screening patients with AML from medical emergency like APL.

Image

Table 1: Pearson Correlation of immunophenotypic markers between HLADR positive and negative AML

	HLA DR -	HLA DR +	P value
CD117	18	20	0.592
CD64	24	23	0.613
CD36	4	9	0.226
CD14	9	11	0.713
CD15	14	18	0.178
CD34	8	23	<u>0.0003</u>
CD7	2	9	<u>0.043</u>
CD19	0	3	0.238
CD56	10	10	0.64

CP-33: Morphology along with peripheral blood flow cytometry can diagnose chronic myelomonocytic leukemia (CMML)

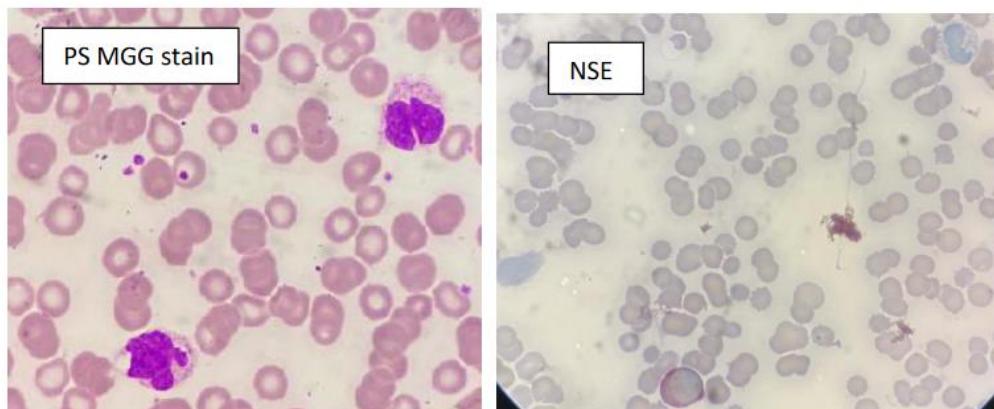
Dr. T Karmakar Roy, Dr. P K Mondal, Dr. S Das, Dr. S Samanta, Dr. S Sengupta, Dr M Bhattacharyay, Dr D Bose

Medica Superspeciality Hospital and Institute of Haematology and Transfusion Medicine, Kolkata

Background: The findings of significant number of abnormal monocytes in peripheral blood and presence of morphological dysplasia are essential in the diagnosis of CMML. But at times, with morphology, after exclusion of myeloproliferative neoplasms and myelodysplastic neoplasms, it is challenging to differentiate CMML from reactive monocytosis, for which flow cytometric evaluation and molecular testing are necessary. Flow cytometry test is widely available than molecular testing and it is also of low cost, but available flow cytometric data on CMML are limited in number.

Objectives: To differentiate CMML from reactive monocytosis by flow cytometric evaluation of peripheral blood. Methods: From February, 2021 to August, 2024 twenty two cases of monocytosis were evaluated by 13 colour flow cytometer - After excluding T, B, NK cells and granulocytes with the help of boolean gating, then monocytes were gated and analysed with CD16 and CD14 and with other antibodies for determining percentage of classical monocytes and aberrant antigen expressions in monocytes, granulocytes and in myeloblasts. Seven healthy control samples were also analysed for collection of baseline data

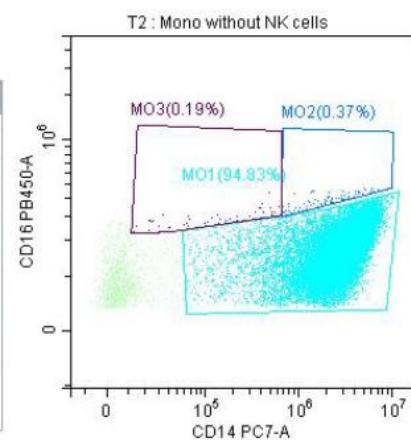
Results: 1) Presence of classical monocytes - Out of total 22 cases, 16 CMML cases show significant increase number of CD14+ve CD16-ve classical monocytes (MO1) ($97.3 \pm 1.8\%$, $P < 0.001$), MO2 $1.9 \pm 0.8\%$, MO3 $0.8 \pm 0.65\%$. Whereas other 6 reactive monocytosis cases show MO1 $79.2 \pm 3.3\%$, MO2 $5.8 \pm 1.6\%$, and MO3 $14.7 \pm 2.3\%$. 2) Aberrant antigen expression in monocytes - In CMML, these classical monocytes show aberrant expression CD56 (96%, $P < 0.01$), CD2 (48%, $P < 0.03$), and decreased intensity of CD11b, CD13 and HLA-DR expression. 3) Aberrant antigen expression in granulocytes - In granulocytes there is decreased side scatter and abnormal pattern in CD11b/CD16/CD13 expression (100%, $P < 0.004$) suggesting presence of granulocytic dysplasia. 4) Aberrant antigen expression in myeloblasts - There are around $1.8 \pm 0.4\%$ CD34+ve myeloblasts with increased CD117 expression (88%), and aberrantly positive for CD7 (74%, $P < 0.03$) and CD56 (100%, $P < 0.001$), and dim positive for CD38 (68%) ($P < 0.06$). Conclusion: Taking 94% cut off value for classical monocytes with presence of its aberrant antigen expression, and abnormal patterns in granulocytes and myeloblasts able to detect most of the cases of CMML (97% sensitivity and 96.7% specificity).



Tube Name: T2

Sample ID: CMML new panel

Population	Events	% Total	% Parent
>All Events	156338	100.00%	100.00%
singlets	107298	68.63%	68.63%
45 pos	89259	57.09%	83.19%
7 neg	62285	39.84%	69.78%
7 & 24 neg	60615	38.77%	97.32%
Mono without NK c...	26428	16.90%	43.60%
MO1	25061	16.03%	94.83%
MO2	98	0.06%	0.37%
MO3	50	0.03%	0.19%



CP-34: NG2 expression pattern and its predictive value for different types of KMT2A translocations (MLL gene rearrangement) in AML

KK Sahoo, : Sweta Rajpal, Sitaram Ghogale, Nilesh Deshpande, Jagruti Patil, Karishma Girase, Gaurav Chatterjee, Nikhil V. Patkar, Papgudi G. Subramanian, Prashant R. Tembhare

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Background Expression of Neuron glial antigen-2(NG2), a flow cytometric marker is known to be associated with KMT2A-rearrangement (KMT2A-r) in acute leukemia that carries poor prognosis. However, positive predictive value(PPV) of NG2 is relatively low, possibly due to heterogeneity in its cut-off value by flow cytometry. The KMT2A(formerly MLL) gene is mapped on chr11q23 and over 100 fusion-partner genes for KMT2A-rearrangement have been identified, among which AFF1, MLLT3, MLLT1, MLLT10, ELL and AFDN are commonly seen.

AIM: To identify association of NG2 expression with any specific type of translocation in KMT2A-r. Materials & Methods The study included 1667 newly diagnosed AML. Multiparametric FCM was performed using a 12-13color panel including antibody against NG2 (clone-9.2.27, FITC) on the DxFLEX and data was analysed using Kaluza software (V2.1.1). Cutoff for NG2 positivity was taken as $\geq 10\%$ of gated blasts. Data was correlated with clinical, cytogenetic and molecular findings.

Results NG2 was positive in 57(3.41%) of 1,667 AML cases. Median age of NG2-positive patients was 13yr(0-67 years). Among these 57 cases, 46 (80.70%) showed KMT2A-r positivity, while 7 (12.28%) were negative. Other abnormalities included one case each of CBFB::MYH11, 5q deletion, two cases of trisomy8, whereas specific partner gene was not identified in three cases, which might be due to cryptic translocation. Among the fusion partners of KMT2A-r, MLLT3 (31.11%) and MLLT10 (20.00%) were the most frequent, with 40% of cases showing unidentified partners. Additionally, we looked for KMT2A cases from cytogenetics data with ELL and all these were found to be negative for NG2.

Conclusions: We concluded that NG2 expression could detect different fusion partners in KMT2A-r-AML, particularly cases involving MLLT3, MLLT10 and MLLT4. We observed complete absence of NG2 expression in KMT2A-ELL fusion. Hence, NG2 may serve as a useful biomarker for predicting the genetic translocation of KMT2A in AML.

KMT2A (n= 46)	MLLT3 (n, %)	MLLT4 (n, %)	MLLT10 (n, %)	Unknown partner (n, %)
NG2 Positive	14 (30.43)	4 (8.69)	9 (19.56)	18 (39.13)

Table 1. NG2 Positivity in KMT2A-Rearranged AML Cases by Specific Fusion Partners

CP-35: Myeloid/lymphoid neoplasm with JAK2 rearrangement:- PCM1::JAK2 fusion presenting as MPN and later transformation to B ALL - a case report.

Dr. Krishna Dev D, Dr. Vishnu Vardhan K, Dr. Wanve Balasaheb Ajinath, Dr. Anuradha Sekaran

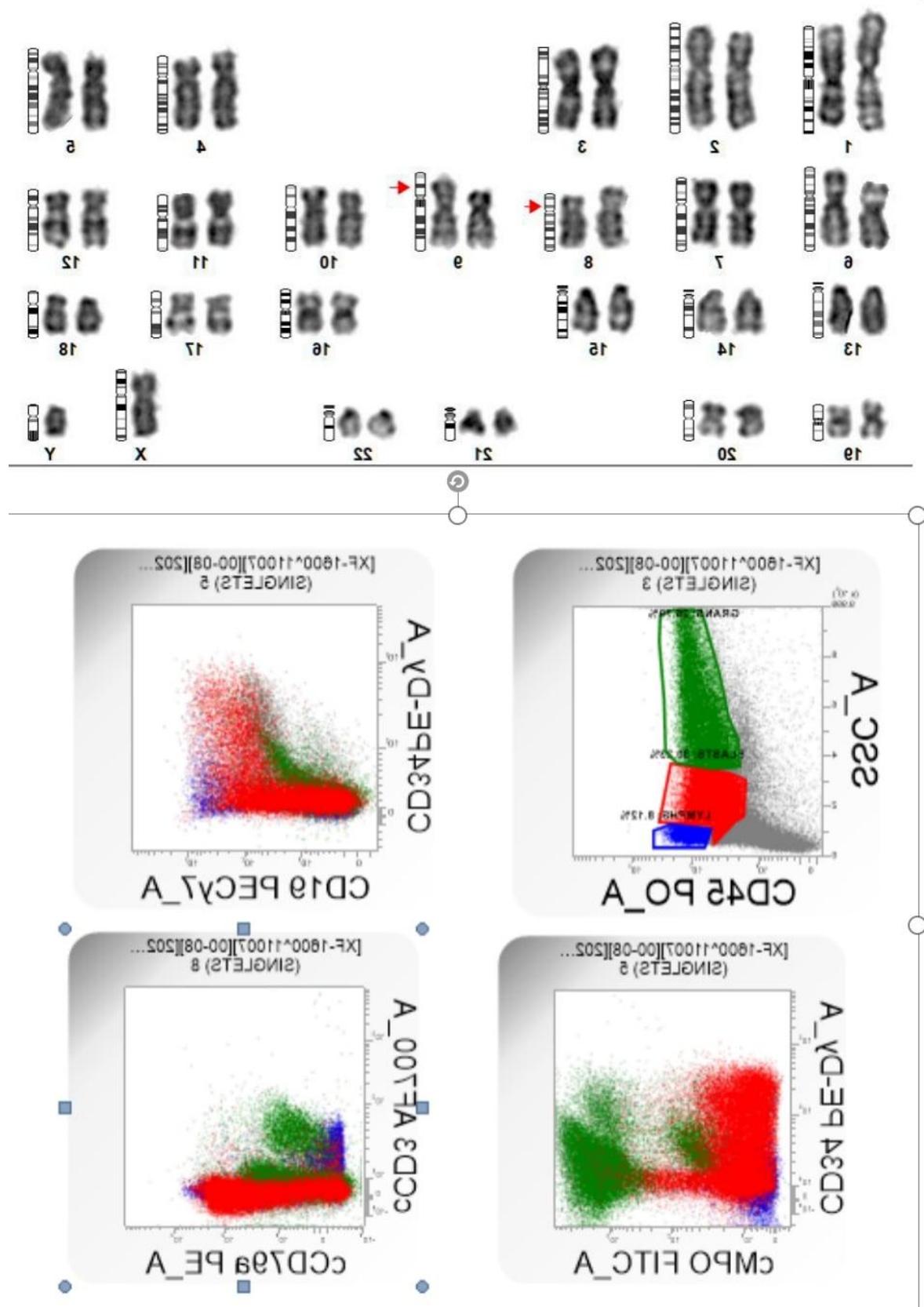
AIG Hospitals, Gachibowli, Hyderabad

Introduction: Myeloid/lymphoid neoplasm with JAK2 rearrangement have the most common fusion partner as PCM1. Patients usually present as chronic myeloproliferative neoplasms (MPN) (i.e., chronic eosinophilic leukaemia, primary myelofibrosis (PMF)) with neutrophilia, monocytosis, eosinophilia, and cytopenias/cytoses.

Methods and Results: A 44-year-old male patient initially presented and was diagnosed elsewhere in 2022 as myeloproliferative neoplasm (myelofibrosis) with bone marrow showing eosinophilia and erythroid hyperplasia. He underwent splenectomy (due to symptomatic splenomegaly); the patient was kept on thalidomide. The MPN molecular panel was negative. Patient recently presented to our hospital with weakness and weight loss. Peripheral blood showed hemoglobin – 7.4 g/dL, WBC count – 104600 cells/cumm, with smear showing leukocytosis with left shift and increase in blasts (09%). Bone marrow aspirate was a dry tap with aparticulate smears, with imprints showing an increase in blasts. Trehine biopsy was hypercellular with nodules/sheets of blasts, eosinophilia, and fibrosis. Flow cytometric analysis revealed B lymphoblasts (40% blasts), suggesting leukemic transformation in a known case of MPN. Concurrent karyotyping showed a PCM1::JAK2 fusion resulting from a t(8;9)(p22;p24) translocation. (46,XY,t(8;9)(p22;p24)[20]). NGS did not reveal any mutations. Discussion: Demonstration of JAK2 rearrangement in the myeloid cells in cases presenting as B-ALL; a prior history of a chronic myeloid neoplasm, or myeloproliferative-like features in the post-treatment setting support a diagnosis of myeloid/lymphoid neoplasms with JAK2 rearrangement, as seen in our case. Prognosis is variable, including a more indolent course in patients presenting with chronic phase disease or a more aggressive course in patients presenting with blast-phase disease. These neoplasms may respond to the JAK2 inhibitor, ruxolitinib, but the response is short-term with relapses.

Conclusion: A multimodal approach is required, including special hematology tests such as flow cytometry, karyotyping, FISH, and molecular assays to accurately classify the neoplasm for prompt and optimal treatment.

Image



CP-36: FxCycle violet based flowcytometry DNA ploidy in patients of B-acute lymphoblastic leukemia – A study Of 118 cases

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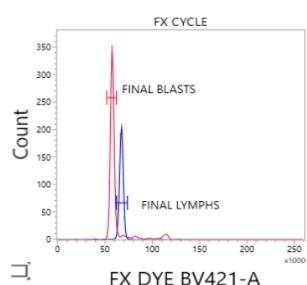
Neuberg Sehgal Path Lab

Background: Role of abnormal DNA ploidy is endorsed as prognostic factor in B- cell acute lymphoblastic leukemia (B-ALL). FxCycle Violet (FCV) based flow cytometric (FCM) DNA ploidy analysis is a simple and expeditious way that can substantiate in characterizing the biological behaviour and is comparable to cytogenetic studies.

Materials and Methods: We performed a prospective study in which FCV based FCM DNA ploidy analysis was done in n=118 new samples of B-ALL. The FCM based DNA ploidy data was compared with corresponding cytogenetic ploidy (conventional karyotyping and/ or FISH) results, wherever available.

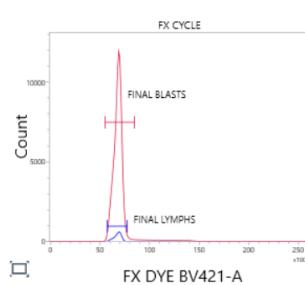
Results: Amongst the FCM ploidy cases (n=118), total number of females were 35 females and 83 males with an age range of 11 months to 66 years. The overall flow-based DNA index (DI) analysis showed diploidy in 57/118 cases (48.3%) (13 females, 44 males), hyperdiploidy in 52/118 cases (44.1%) (18 females and 34 males), and hypodiploidy in 9/118cases (7.6%) (4 females and 5 males). Cytogenetic based ploidy was available only in 82/118 cases (69.4%) with a significant number of cases showing culture failure. The overall cytogenetic analysis showed diploidy in 39 / 82 cases (47.6%) (females 10, males 29), hyperdiploidy in 35/82 cases (42.7 %) (12 females and 23 males), and hypodiploidy in 8/82cases (9.7%) (5 females and 3 males) A detailed analysis of concordance and discordant cases and their reasons will be discussed in the main presentation Conclusion: FCV-based ploidy is a sensitive technique that provides complementary information and ascertains a strong correlation with conventional cytogenetics in cases of B - ALL. Its edge over cytogenetics lies in early reportability, ability to detect endoreduplication in all B-ALL and to process even haemodiluted samples in which there is cytogenetic culture failure.

Flow Cytometry based DNA Ploidy analysis using Fx Cycle violet dye



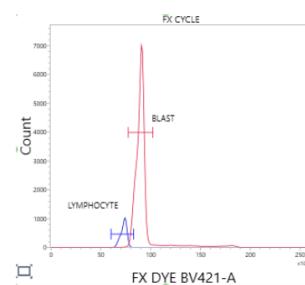
DNA PLOIDY = Hypodiploid

DNA INDEX= 0.85



DNA PLOIDY = Diploid

DNA INDEX= 1.005



DNA PLOIDY = Hyperploid

DNA INDEX= 1.22

CP 37: CD26+ Leukemic Stem Cell Identification by Flow Cytometry: Role in Diagnosis and Follow-up of Chronic Myeloid Leukemia

Namrata Kaul, Praveen Sharma, Man Updesh Singh Sachdeva, Shano Naseem, Sreejesh Sreedharanunni, Parveen Bose, Arun Kumar, Bhavishan Thakur, Pankaj Malhotra

Postgraduate Institute of Medical Education and Research, Chandigarh.

Introduction

Recent investigations have explored the potential of CD26+ leukemic stem cells (LSCs) as a diagnostic marker in chronic myeloid leukemia (CML). This study aimed to assess the diagnostic significance of CD26+ CML LSCs using flow cytometric immunophenotyping.

Methods Patients over 12 years old with clinical suspicion and a morphological diagnosis of CML were included in the study. We used peripheral blood (PB) and bone marrow (BM) samples for the enumeration of CD26+ LSCs. A pre-titrated antibody cocktail containing CD45, CD34, CD38, and CD26 monoclonal antibodies was prepared. Control samples were obtained from patients with non-CML conditions, including non-CML myeloproliferative neoplasms (MPNs), acute leukemias, MDS/MPNs, and reactive bone marrows. Reverse Transcriptase (RT)-PCR was performed to identify the BCR::ABL1 transcript type in all cases, and fluorescence in situ hybridization (FISH) was conducted on a subset of cases to analyze BCR::ABL1 positivity in sorted CD26+ LSCs.

Results A total of 218 samples were tested, including 177 PB and 41 BM samples. The cohort comprised 72 patients with chronic phase CML (CML-CP), 4 patients in the accelerated phase (CML-AP), 7 in blast crisis (CML-BC), 15 follow-up CML patients, and 79 non-CML cases. CD26+ LSCs were found in 100% of patients with a confirmed BCR::ABL1 fusion. There was a strong correlation between CD26+ CML LSCs in the PB and BM ($r = .917$). Notably, follow-up CML patients with negative RT-PCR results did not show any CD26+ LSCs.

Conclusion The consistent detection of CD26+ LSCs in all CML cases, combined with its cost- effectiveness and speed, suggests that PB flow cytometric estimation of CD26+ LSCs could serve as a promising surrogate for molecular genetic techniques at the time of diagnosis. Its role in assessing the ‘stem cell response’ during follow-up needs further exploration.

CP-38: IMMUNOPHENOTYPING IN TRANSIENT ABNORMAL MYELOPOIESIS ASSOCIATED WITH DOWN'S SYNDROME: A CASE SERIES

Lalita Jyotsna Prakhyaa, LADY HARDINGE MEDICAL COLLEGE, NEW DELHI

Background: Transient Abnormal Myelopoiesis (TAM) is a haematological condition frequently associated with Down syndrome (DS), characterized by somatic mutations in the GATA1 gene. This case series investigates three TAM cases in DS patients diagnosed between 2023 and 2024.

Methods: We conducted a retrospective review of clinical data for three DS patients with TAM, aged 10 days to 3 months. Clinical presentations, peripheral smear findings, immunophenotyping results, and outcomes were analyzed.

Results: Case 1: A 3-month-old female with hepatosplenomegaly and 30% blasts on peripheral smear. Immunophenotyping showed positivity for HLA-DR, CD117, CD13, CD33, CD61, CD41, CD71, and aberrant CD7 and CD4. Case 2: A 10-day-old female presented with fever, hyperleukocytosis, and thrombocytopenia. Peripheral smear revealed 21% blasts. Immunophenotyping was positive for CD34, CD117, MPO, CD33, CD61, CD41, CD36, CD71, and aberrant CD7. Case 3: A 25-day-old male with dyspnea, cyanosis, pericardial effusion, and hepatosplenomegaly. Peripheral smear showed 30% blasts. Immunophenotyping was positive for CD34, HLA-DR, CD117, CD15, MPO, CD61, CD41, CD36, CD71, and aberrant CD7. Cytarabine was given in view of hyperleukocytosis and significant clinical presentation. On follow up, all patients improved clinically with receding leucocyte counts and blast counts and were kept on follow-up. Conclusion: TAM in DS presents with diverse clinical and laboratory features. Key immunophenotyping markers include CD34, HLA-DR, CD117, CD13, CD33, CD41, CD61, CD71, and CD7.

Image

CASE SERIES			
Case	Case 1	Case 2	Cases 3
Clinical features	3months w ith HSM and with DS phenotype	10day baby with fever, leucocytosis, Thrombocytopenia and DS phenotype.	5day baby with dyspnea,cyanosis, pericardial effusion and HSM with DS phenotype.
CBC PS	TLC-7.42/uL,PLT-221000/uL Hb- 5.7 with 30% blast in PS	TLC-26.77/uL PLT-35000/uL,Hb- 11.9 21% blasts in PS	TLC-142.3X103/uL PLT-12000/uL Hb-10.3.30% blasts in PS
IPT	HLA-DR,CD117CD13, CD33 CD61,CD41,CD71 POSITIVE Aberrant CD7,CD4 Positive	CD34,MPO,CD117 CD33,CD61,CD41 CD71,CD36,CD56 Aberrant CD7 Positive	CD34,HLA-DR CD117,MPO POSITIVE CD33,CD15,CD61, CD41 CD71,CD36 Aberrant CD7 Positive
Follow up	Allpatients improved clinically with receeding leucocyte andblast counts and w ere kept on follow-up.		

CP-39: Genetic landscape of Acute Myeloid Leukemia with downregulation of CD38 median fluorescent intensity in Leukemic blasts

Shreyam Acharya, Richa Chauhan, Jasmita Dass, Preeti Khokhar, Ganesh KV, Mukul Aggarwal, Pradeep Kumar, Rishi Dhawan, Tulika Seth, Seema Tyagi, Manoranjan Mahapatra

All India Institute of Medical Sciences (AIIMS), New Delhi

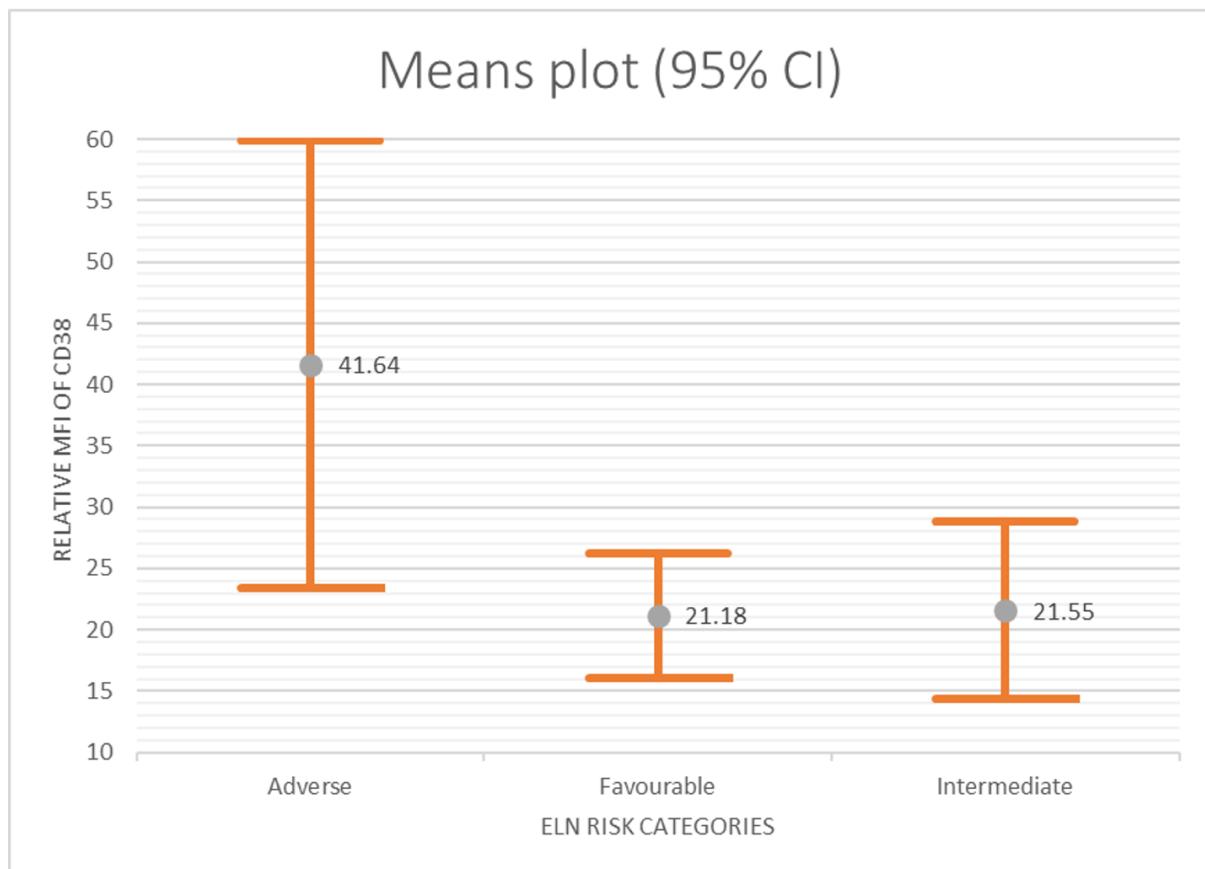
Background: Myeloid blasts in Acute myeloid leukemia (AML) can express variable CD38. We hypothesised that a reduced expression of CD38 in AML blasts may harbour a higher burden of leukemic stem cells that can be related to an unfavourable genetic landscape in these patients.

Method: A total of 79 newly diagnosed patients recruited as per convenience sampling were included. Cases whose cytogenetics and molecular data were not available were excluded. Flow cytometric immunophenotyping was performed on BD FACS Cantoll/ FACS Lyric, and 8 to 10 color multiparametric assays were performed. The flow cytometry data was analysed to look for median fluorescent intensity of CD38 on the leukemic blasts and plasma cells. The CD38 relative mean fluorescence intensity (CD38 rMFI) was calculated based on CD38 expression on plasma cells to blasts for normalisation. Also, the difference amongst European LeukemiaNetwok (ELN) risk groups and amongst genetic subtypes was assessed.

Result: Amongst the 79 cases, the CD38 rMFI of leukemic blasts ranged from 3.6-136.6. The cut off for the highest 25% of the CD38 rMFI at the third quartile of 75% was 30.21. 20/79 cases had values of CD38rMFI higher than cut-off. The mean (95% CI) of adverse ELN subgroup (41.64; 95% CI of 23.36-59.91) was significantly higher than intermediate (21.55, 95% CI of 14.31-28.77) or favourable risk group (21.18; 95% CI of 16.10-26.26) with a p value of 0.0045. The CD38 rMFI was documented to be higher in 100% cases (n=4) with complex karyotypes. Driver mutations JAK2 (n=1), CBL (n=1), PTPN11(3/4) and U2AF1 (n=3/4) were associated with higher rMFI. All TP53 mutated cases (n=2) showed higher rMFI, while all cases with normal molecular workup showed low rMFI (n=5). 95% (17/18) of AML with RUNX1::RUNX1T1 had low rMFI as were almost all (n=15) NPM1 mutated AML except when associated with WT-1 (n=2).

Conclusion: A higher CD38 rMFI of leukemic blasts, as evidenced with significantly higher value in the adverse ELN subgroup in AML may be associated with an unfavourable genetic landscape and treatment outcome. We observed higher prevalence of complex karyotype, TP53 mutation, driver mutations such as CBL, U2AF1, JAK2, PTPN11, and WT1 amongst cases with high CD38rMFI. Almost all AML with RUNX1::RUNX1T1 and NPM1 mutated AML showed normal CD38 rMFI. Larger studies for unravelling the mutational landscape of AMLs with reduced CD38 expression, may have a predictive role in the initial assessment of patients, especially in resource-limited settings.

Image



CP-40: A Human-in-loop Model of Machine Learning Algorithm to Detect T-ALL MRD with Increased Confidence

Phaneendra Datari, Kotteeswari Kathirvel, Mohammed Aakif, Uday Prakash Kulkarni, Anu Korula, Arun Kumar Arunachalam, Vikram Mathews
Christian Medical College - Vellore

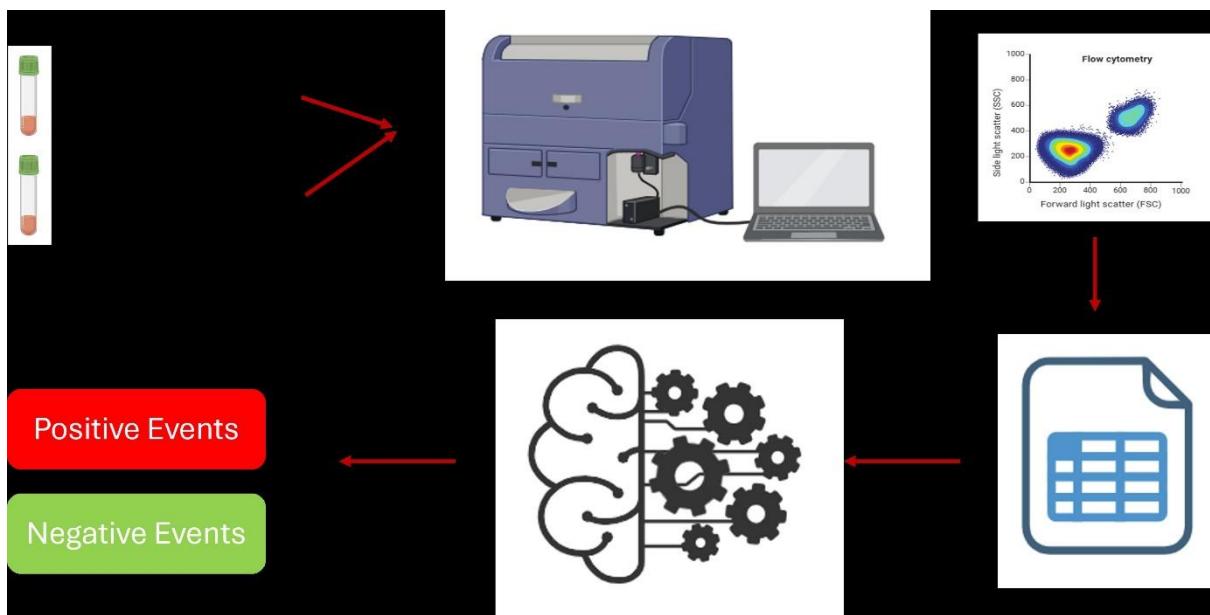
Introduction: Measurable Residual Disease (MRD) is an important biomarker in prognosticating T-cell acute Lymphoblastic leukaemia (T-ALL), as molecular or gene expression-based classification and prognostication is not frequently employed. The lack of LAIPs and well-defined maturation patterns in T-cell precursors make the MRD assessment by FCM more challenging. This study aims to identify the normal precursor T cells and their maturation pattern. We also attempt to employ machine learning to differentiate these normal populations from the abnormal populations confidently.

Methodology: Peripheral blood from 5 normal controls was analysed using an MRD panel comprising CD2, sCD3, cCD3, CD4, CD5, CD7, CD8, CD10, CD16, CD38, CD45, CD48 and CD56. The model was trained using normal controls, with 4 cases with MRD negativity and 5 cases with MRD positivity using standard FCM assessment. This model was then validated on a validation cohort of 91 T-MRD samples that were processed between January and September of 2024.

Results: The gating strategy to evaluate precursor T-cells was – Singlets>Viable cells>CD7+ cells>cCD3+ & sCD3- cells. These cells were evaluated for their expressions of markers like CD2, CD5, CD4, CD8, CD25, CD44 and TRBC1. The maturation pattern of T cells was – CD2-CD5->CD2-CD5dim>CD2+CD5+. The early CD2-CD5- cells were CD44+, CD25-/, CD4-, CD8-, CD48-, CD38-, CD45 dim and TRBC1 non-rearranged. CD2-CD5dim cells were CD44-, CD25dim to negative, CD4+/-, CD8+/- and showed TRBC1 rearrangement. CD2+CD5+ cells were like mature T cells. CD2+ CD5- cells were positive for CD16+56 and are NK-T cells. The ML model was trained on 10,46,400 events from 14 samples. Each event was labelled as normal or abnormal. Among Logistic Regression, Random Forests and XG Boost models, the XG Boost model performed the best with 99% accuracy and a 100% recall rate. This model was then validated with the validation cohort consisting of 91 samples. Concordance with standard FCM assessment was 82%, with discrepancies in 15 cases. These cases were positive by standard assessment but were negative by the ML model. 3 of these cases were with sCD3+ disease, which was excluded in the gating strategy of training the model. Another 7 cases had MRD values of less than the clinically significant cut-off. The ML model had a high PPV of 100%. Overall, the ML model had balanced precision and recall with an F1 score of 74%.

Conclusion: Knowing the normal precursor compartment of T-cells avoids false positive calls of MRD assessment. Incorporating ML algorithms in the general workflow imparts confidence and acts as a double-check in MRD assessments. Feature engineering and hyper parameter tuning will be tried in the next phase to increase the sensitivity of this model.

Image



CP-41: DNTs have a high negative predictive value but a low positive predictive value for the diagnosis of ALPS.

Gayathri Kuppusamy, Mohammed Aakif, Phaneendra Datari, Arun Kumar Arunachalam, Sushil Selvarajan, Uday Prakash Kulkarni, Anu Korula, Biju George, Aby Abraham, Vikram Mathews.

CHRISTIAN MEDICAL COLLEGE, VELLORE

Introduction: Autoimmune Lymphoproliferative Syndrome (ALPS) is a rare genetic disorder characterized by chronic lymphoproliferation and autoimmunity. One of the hallmark features of ALPS is the presence of elevated levels of Double Negative T cells (DNTs), which lack CD4 and CD8 surface markers. The criteria for diagnosing ALPS was first established in 1999 and later modified in 2009, consisting of two required and six accessory criteria. DNTs, one of the required criteria, is often mistaken to be a pathognomonic feature of ALPS. In this study, we aim to evaluate the predictive value of DNTs in diagnosing ALPS.

Methods: All the samples received for the Primary Immunodeficiency screening panel in the Department of Haematology, Christian Medical College, Vellore, between January 2022 and December 2023, were included in the study. Demographic details, clinical details and genetic details were retrieved from patients' electronic medical records.

Results: 387 cases were screened for PID by flow cytometry (FCM) with a median age of 7 years and a male-to-female ratio of ~1. Of these, 168 samples were received with a clinical query of ALPS. Increased TCRab+ double negative T cells (DNTs) were seen in 103/168 cases and were absent in 65/168 cases. As per the 2009 criteria, the required criteria were satisfied in 27/103 cases, 1/27 had a primary accessory criterion, and 25/27 had secondary accessory criteria. Of the 103 with DNTs, 69 had genetic testing performed at our centre. 42/69 showed variants – 22 VUS and 20 Pathogenic/ Likely pathogenic. Of the 20 significant variants, only 7 were related to ALPS and ALPS-like diseases. Among the 65 without DNTs, 31 had genetic data – 17 showed variants – 3 VUS and 14 pathogenic/ likely pathogenic variants. Of the significant variants, only 2/14 showed ALPS-like mutations. With the current cut-off values of DNTs being >1.5% of total lymphocytes and 2.5% of CD3+ lymphocytes, it had a sensitivity of 84.6% in diagnosing ALPS/ ALPS-like diseases. In comparison, the specificity was only 33%. PPV was 15.9% and NPV was 93.5%.

Conclusion: An increased percentage of TCRab+ double negative T cells has a good negative predictive value and sensitivity in diagnosing ALPS but very poor specificity and positive predictive value.

Image

Revised diagnostic criteria for ALPS

Required

1. Chronic (> 6 months), nonmalignant, noninfectious lymphadenopathy or splenomegaly or both
2. Elevated CD3⁺TCRαβ⁺CD4⁻CD8⁻ DNT cells ($\geq 1.5\%$ of total lymphocytes or 2.5% of CD3⁺ lymphocytes) in the setting of normal or elevated lymphocyte counts

Accessory

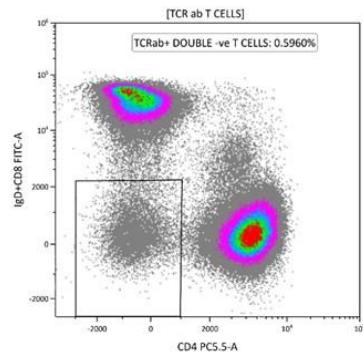
Primary

1. Defective lymphocyte apoptosis (in 2 separate assays)
2. Somatic or germline pathogenic mutation in *FAS*, *FASLG*, or *CASP10*

Secondary

1. Elevated plasma sFASL levels (>200 pg/mL) OR elevated plasma interleukin-10 levels (>20 pg/mL) OR elevated serum or plasma vitamin B₁₂ levels (> 1500 ng/L) OR elevated plasma interleukin-18 levels > 500 pg/mL
2. Typical immunohistological findings as reviewed by an experienced hematopathologist
3. Autoimmune cytopenias (hemolytic anemia, thrombocytopenia, or neutropenia) AND elevated immunoglobulin G levels (polyclonal hyperimmunoglobulinemia)
4. Family history of a nonmalignant/noninfectious lymphoproliferation with or without autoimmunity

A definitive diagnosis is based on the presence of both required criteria plus one primary accessory criterion. A probable diagnosis is based on the presence of both required criteria plus one secondary accessory criterion.



Genes with variants identified in DNT+ cases in our cohort

	GENES
PATHOGENIC / LIKELY PATHOGENIC	<i>IL12RB1, RFAXP, DDX41, ABCD1, SAMD9L, NFKB2, LRBA, STAT3, LYST, STXBP2, IL12B, MVK, ADA2, KRAS, CIITA, IL12RB1, MLH1, VWF</i>
VUS	<i>CYBA, IRAK1, TCIRG1, VPS13B, CDAN1, CACNA1S, KMT2D, FOXP3, MVK, CHD7, CFHR3, IKZF1, MYH9, PRF1, NFKB2, PRKCD, FASLG, RAG1, KMT2D, MCR2, TCF3, MEFV, FAS, ADA, G6PD, VWF</i>

CP 42: Utility of CD180 in Classification and Identification of B-Cell Non-Hodgkin Lymphomas

Merlin Priyanka, Krishna Chaitanya, Phaneendra Datari, Arun Kumar Arunachalam, Gayathri Kuppusamy, Anu Korula, Sushil Selvarajan, Uday Prakash Kulkarni, Biju George, Aby Abraham, Vikram Mathews.

CHRISTIAN MEDICAL COLLEGE

Introduction: CD180, also known as RP105 (Radioprotective 105), is a toll-like receptor (TLR) family member primarily involved in B-cell activation and innate immune response(1). Studies have shown that over-expression of CD180 is associated with Marginal Zone Lymphomas (MZL), and under-expression, in association with under or over-expression of other markers, is associated with Chronic Lymphocytic Leukaemia (CLL) and Mantle Cell Lymphoma (MCL). In this study, we aim to evaluate the expression of CD180, as a single biomarker, among the types of B-NHLs and its utility in the classification of B-NHLs.

Methods: We analysed the CD180 expression in the cases of B-NHLs diagnosed at the Department of Haematology, Christian Medical College, Vellore, between June 2023 and June 2024. Wherever available, bone marrow reports and other clinical details were retrieved from the patients' electronic medical records.

Results: A total of 165 cases were included in the study, with a median age of 57 years and a male-to-female ratio of 2.7. Of the 165, CLL were 77, MCL were 18, MZL were 17, Diffuse Large B-cell Lymphomas (DLBCL) were 15, Hairy Cell Leukaemia (HCL) were 8, Burkitt Lymphoma (BL) were 5, Follicular Lymphoma(FL) were 4, Lymphoplasmacytic Lymphomas (LPL) were 3, small cell B-NHL – unclassified were 15 and large cell B-NHL – unclassified were 3. Under-expression of CD180 was seen in 121/165 (73%) cases, over-expression was seen in 12/165 (7%), and equivalent expression was seen in 32/165 (20%). Among the underexpressors, CLL>MZL>DLBCL and among the overexpressors HCL=Low-grade NHL>MZL=DLBCL. Equivalent expression was predominantly seen in MCL. Under expression of CD180 had a positive predictive value(PPV) of 87% in detecting CLL among CD5+ B-NHLs. Among MZL, under-expression of CD180 was seen in 12/17 cases, over-expression was seen in 2/17 cases and the rest showed equivalent expression. CD180 did not show any significance in classifying other types of B-NHL in our study.

Conclusion: Under-expression of CD180 is a helpful marker in identifying B-NHL. Among CD5+ B-NHLs, under-expression of CD180 has a PPV of 87% in identifying CLL.

CLL v/s MCL

	CLL	MCL
CD180 DOWNREGULATED	66 (TRUE POSITIVE)	10 (FALSE POSITIVE)
CD180 MODERATE	10 (FALSE NEGATIVE)	8 (TRUE NEGATIVE)

POSITIVE PREDICTIVE VALUE OF DOWNREGULATION OF CD180 PREDICTING CLL OVER MCL WHEN BOTH CLL AND MCL ARE CONSIDERED AS DIFFERENTIALS IS 86.8%

CP 43: Acute myeloid leukaemia with RUNX1::RUNX1T1 fusion versus Mixed-phenotype acute leukaemia, B/myeloid : A diagnostic conundrum in immunophenotyping

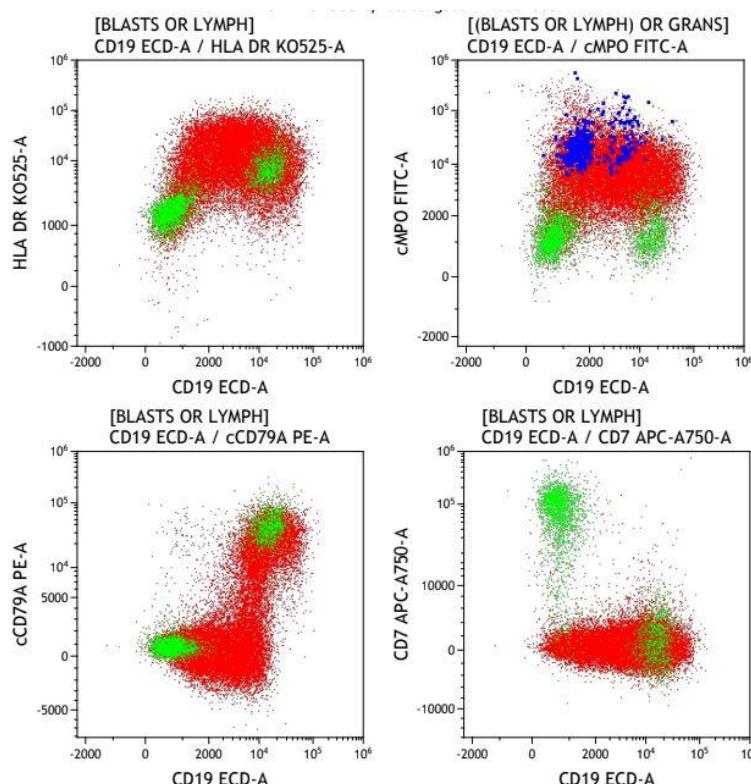
Lumen Agarkar, Prabhu Manivannan

Jawaharlal Institute of Postgraduate Medical Education & Research (JIPMER)

Case report: A 28-year-old woman with a history of acute myeloid leukemia (AML) and one previous relapse presented with fever, fatigue, and headaches lasting two weeks. Imaging revealed an intracranial bleed. Records from her prior treatment are unavailable. On further investigation, 86% of blasts were in the peripheral blood and 89% in the bone marrow. The bone marrow aspirate was subjected to immunophenotyping, which revealed 70.31% of all events under CD45 moderate and low side scatter region. These events were strongly positive for CD34, HLA-DR, cMPO, CD19, cCD79a, CD38, and CD58, with variable positivity for CD117, CD33 and CD81 and weak positive for CD11c. Hence, it was reported as Mixed phenotype acute leukemia, B/myeloid. However, further cytogenetic testing showed the t(8;21). She was started on a FLAG plus venetoclax regimen.

Discussion: It is not uncommon for AML with t(8;21) to exhibit B-marker expression. Incorporating additional cytogenetic and molecular studies is essential for accurate diagnosis and classification of AML. The t(8;21) translocation represents a distinct entity and is well-known for its favorable prognosis. In contrast, MPAL-B/M is generally considered high-risk acute leukemia, with outcomes that fall between those of acute myeloid leukemia (AML) and B-lymphoblastic leukemia despite intensive treatment. Whether this case must be labeled as MPAL or considered AML with a possible t(8;21) translocation remains a dilemma in a resource-limited setting. The possibility of t(8;21) AML progressing to MPAL also arises in such cases.

Image



CP-44: Early T precursor acute lymphoblastic leukemia - A case series

Syed Taha Hussain, Manasi Mundada, Faiq Ahmed, Suseela K, Kinjal Shah, Rohan Tewani, Pavankumar B, Veerendra Patil

Basavataramak Indo American Cancer Hospital and Research Institute, Hyderabad

Introduction: Early T precursor acute lymphoblastic leukemia (ETP-ALL) is a neoplasm composed of blasts committed to the T-cell lineage with a unique immunophenotype that includes expression of stem cell markers and/or myeloid lineage markers. The diagnosis and management of ETP-ALL is still challenging, especially for relapse/refractory disease.

Aims and objectives: To study the prevalence, clinicopathological characteristics and immunophenotypic profiles of cases diagnosed as ETP-ALL and their follow up.

Material and Methods: Twenty nine newly diagnosed cases of ETP-ALL between Jan 2021 to Feb 2024 were included in the study. The relevant clinicopathological and follow up measurable residual disease(MRD) data was retrieved from the files archived at BIACH & RI, Hyderabad. Flow cytometry was done using CD45, CD34, HLADR, CD19, CD79a, CD10, CD1a, CD2, sCD3, cCD3, CD4, CD5, CD7, CD8, CD56, cytoMPO, CD33, CD13 and CD117 panel on Beckman DxFlex with Kaluza software version 2.1.

Results: The age ranged from 8 to 63 years with a median age of 21 years. There was male predominance with a male to female ratio of 3.1:1. Leucocytosis was seen in 20 cases (68.9%) and leucopenia in 9 cases (31%). Lymphadenopathy was seen in 16 cases (55.1%) and hepatosplenomegaly in 4 cases (13.7%). Hemoglobin levels ranged from 3.9g/dl to 11.4g/dl (Median : 7.5 g/dl). Total leucocyte count ranged from $0.2 \times 10^3/\mu\text{l}$ to $192.5 \times 10^3/\mu\text{l}$ (Median : $35 \times 10^3/\mu\text{l}$). Platelet count ranged from 6000 to 397000 / μL (Median : 40000/ μl). Blasts in peripheral blood ranged from 2% to 92%. Flow cytometry was done on peripheral blood/bone marrow aspirate sample. Immunophenotyping showed ETP-ALL profile in 26 cases and Near ETP-ALL profile in 3 cases. Follow up post induction (BFM-95 protocol) MRD data was available in 14 cases of which 7 cases had a positive MRD (7/14, 50%). Serial follow up data would be presented in detail.

Conclusion: Identification of ETP-ALL is important for their therapeutic and prognostic connotations.

CP-45: Exploring CD36 Expression in Adult B-acute lymphoblastic leukemia: Insights into Genetic Risk and Treatment Response.

Satyender Dharamdasani¹, Hari Neupane¹, Sreejesh Sreedharanunni¹, Nabhajit Mallik¹, Shano Naseem¹, ManUpdesh Sachdeva¹, Alka Khandelwal²

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2. Department of Clinical Hematology and Medical Oncology, PGIMER, Chandigarh.

Background: CD36, a surface glycoprotein commonly found on platelets, monocytes, and erythroid progenitor cells, is less frequently expressed on B-lymphoblasts. We investigated the frequency of CD36 expression in adult B-cell acute lymphoblastic leukemia (B-ALL) blasts and its association with clinical, immunophenotypic, genetic features, and minimal residual disease (MRD) status.

Methods: We retrospectively analyzed 647 adult B-ALL cases diagnosed at our institute between October 2018 and September 2023. Clinical laboratory parameters, bone marrow findings, immunophenotyping results, cytogenetic and molecular genetic profiles, and MRD levels from flow cytometry were recorded and evaluated.

Results: Our cohort included 647 adult B-ALL cases with a mean age of 41 years (range: 14–78 years) and a male-to-female ratio of 1.6:1. CD36 expression was observed in 43/577 cases (7.45%). CD36- positive cases exhibited significantly less severe thrombocytopenia, with a median platelet count of 41,000/ μ L (IQR: 19,000–98,000) compared to 24,000/ μ L (IQR: 12,000–57,000) in CD36-negative cases ($p=0.05$). Leucopenia was present in 21.8% of CD36-negative cases compared to 9.3% of CD36-positive cases ($p=0.05$). No significant differences were observed in hemoglobin levels, total leukocyte counts, or peripheral blood blast counts. Immunophenotypic analysis revealed that CD36 expression was significantly associated with CD20 (79% in CD36-positive vs 61.5% in CD36-negative cases, $p=0.02$) and CD123 positivity (57.5% vs 39.7%, $p=0.04$) in leukemic B-blasts. CD36 expression was also linked to the presence of the BCR::ABL1 p210 transcript (76.5% in CD36-positive vs 45.9% in CD36-negative cases, $p=0.02$). High-risk genetic features, such as BCR::ABL1, BCR::ABL1-like, and KMT2A rearrangements, were more prevalent in CD36-positive cases compared to CD36-negative cases (75% vs 32.7%, $p=0.015$). Furthermore, CD36-positive cases showed a higher rate of end-of-induction MRD positivity (77.3% vs 40.7%, $p=0.001$).

Conclusion: CD36 expression in adult B-ALL was significantly associated with distinct laboratory, genetic, and MRD response profiles, highlighting its potential role as a prognostic marker.

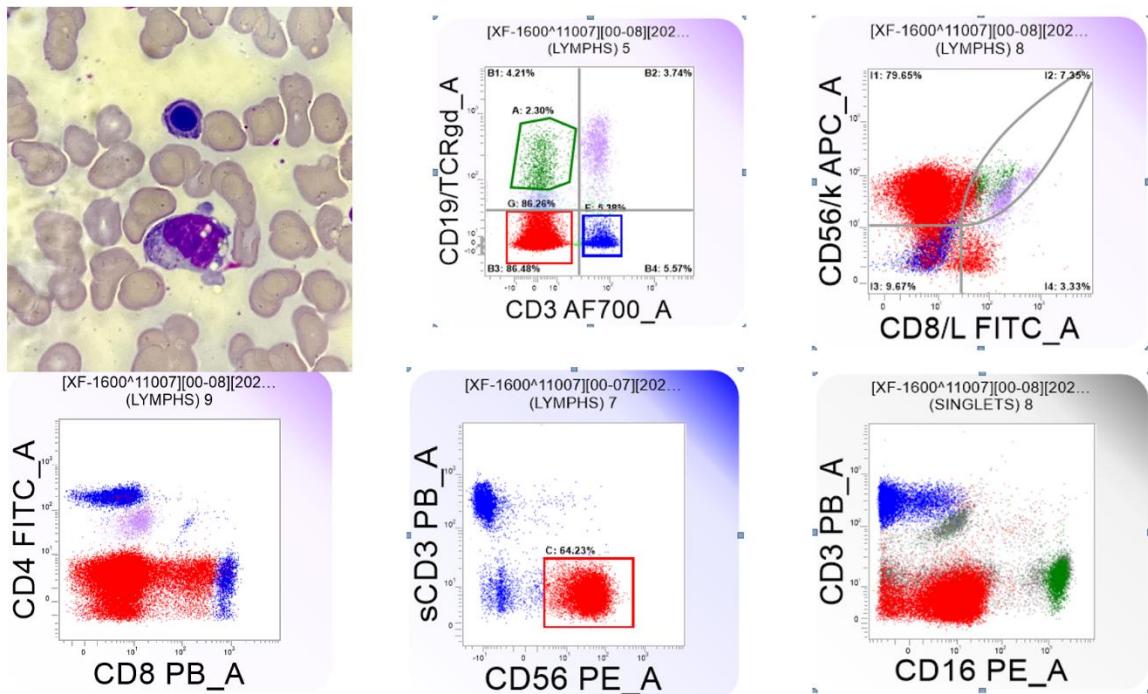
CP-46: Natural Killer-large granular lymphocytic leukaemia (NK-LGLL) - a case report

**Dr. Krishna Dev D, Dr. Sara Razzak, Mr. Suresh Kotrakona, Dr. Wanve Balasaheb Ajinath,
Dr Anuradha Sekaran
AIG Hospitals.**

Abstract

NK-large granular lymphocytic leukaemia (NK-LGLL) is a neoplasm with persistent increase in peripheral blood NK cells (greater than $2 \times 10^9 / L$) in the absence of a clearly identifiable cause and a chronic indolent clinical course. NK-LGLL comprises of 2-5% of chronic lymph proliferative disorders. Our case was a 63 year old female, presented with cytopenias, hematochezia, malena, weight loss in december 2023. Previously she was diagnosed as tuberculosis and was on antitubercular therapy (ATT) in 2020. Blood investigations in december 2023 showed anemia, Hb-9.5g/dL and leucopenia (WBC- 2130 cells/cumm). Bone marrow was reported as mildly hypocellular marrow with ill defined granulomas. Flow cytometry revealed - a clone of NK cells (20%) with CD4, CD8 -ve, CD56+. Patient was kept on anti tubercular therapy in view of granuloma on marrow. Patient came for follow up, in september 2024, with blood counts of WBC - 5200 cells/cumm, with lymphocytes - 72% on differential and hemoglobin of 12 g/dL. Peripheral smear showed increased large granular cells with round to irregular nuclei with condensed chromatin, irregular nuclear contours and moderate granular cytoplasm. Flow cytometry was done revealing 65% of NK cell population with CD56+, CD16 variable+, CD38+, HLA-DR variable +, sCD3-, CD4-, CD8 -, CD5-. Sample was sent for NK cell clonality flow testing, which revealed loss of KIR markers within the abnormal NK cell population with CD94 overexpression, confirming the clonal nature of the NK cell proliferation. In view of chronic clinical history, blood counts and flow cytometry findings, diagnosis of Natural Killer-large granular lymphocytic leukaemia (NK-LGLL) was given. Treatment for the patient was planned with cyclosporine/ methotrexate, response to be assessed. NK-LGLL though usually a chronic disease, it is important to identify because it leads to neutropenia leading to recurrent infections. Cytopenias and recurrent infections indicate a worse prognosis in these patients as seen in our case.

Image



CP-47: Immune reconstitution after CD19 CAR-T cell therapy in patients with relapsed/refractory B cell Acute lymphoblastic leukemia/lymphoma (r/r B-ALL/L)

Mohammed Yasar, Hamenth Kumar Palani, Arun Kumar Arunachalam, Phaneendra Datari, Sushil Selvarajan, Uday Kulkarni, Anu Korula, Biju George, Aby Abraham, Vikram Mathews

Christian Medical College

Background: Immune reconstitution following Chimeric Antigen Receptor (CAR) T cell therapy in r/r B-ALL/L is crucial in shaping the overall immune response and long-term efficacy.

Methods: Ten patients (n=6 r/r B-ALL; n=4 DLBCL) with a median age of 45 (range 6-59) were enrolled. Autologous CAR T cells targeting CD19 (LTG1563; Miltenyi Biotec) were infused under a dose escalation strategy (0.5-2x10⁶ cells/kg; 3+3+4). Circulating lymphocytes, T cell subsets - CAR+, CAR- T cells and memory subsets were evaluated in peripheral blood samples using flow cytometry with a standardized antibody panel at specified time points. Data analysis was done using Kaluza 2.1 (Beckman), and GraphPad Prism (v8.0.1).

Results: Median T cell transduction efficiency (CAR positivity) was 38% (range 16-55). Total T cell normalized by day 14 (range 14-28). Median CAR+ T cell expansion was observed on Day 14 (range 14-28). Among CAR+ T cells, rapid recovery was seen in CD8+ T cells compared to CD4+ T cells, resulting in a CD4/CD8 ratio <1.0. Subset analysis revealed a rapid expansion of central memory T cells initially followed by a robust expansion of effector memory T cells. CAR-T cell subset followed the same recovery and memory subsets pattern. In the innate compartment, NK cells were first to recover, returning to normal levels within 30 days (range, 14-28). B cell aplasia persisted in all patients on early assessment (day 7), B cells were detected in 1/10 (10%) on day 14 but remained below the normal level until last follow up in all remaining patients.

Conclusion: This study shows that immune reconstitution after CAR T cell therapy follows a distinct recovery pattern among different cell types. While overall normalization of various cell types occurred earlier in r/r B-ALL patients compared to DLBCL, earlier NK cell recovery was observed in all patients. Prolonged B cell aplasia in most patients indicates the ongoing CAR T cell function

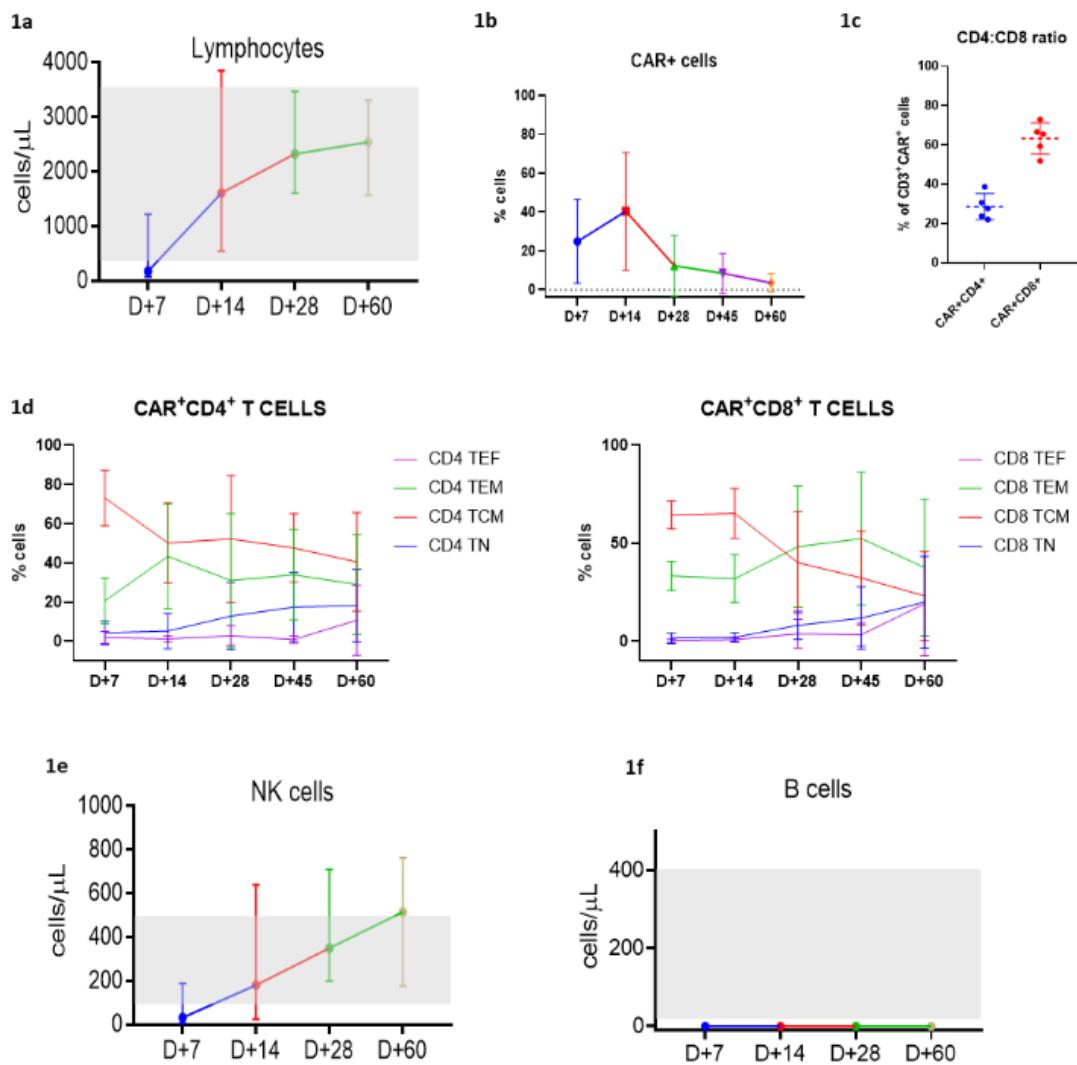


Figure 1. Profile of immune reconstitution after anti-CD19 CAR-T cell therapy. A) Recovery of Total lymphocyte counts post CAR T cell infusion. B) Distribution, expansion, contraction and persistence of CAR T cells. C) Inverted CD4/CD8 ratio among CAR+ T cells indicating sustained immune response. D) Subset analysis via multi-parameter flow cytometry using CD45RO and CD62L. CD8+ T cells showing an inverse relationship in expansion between central and effector memory phenotype. E) Rapid NK cell recovery and transient increase in cell numbers thereafter. F) B cell kinetics, indicated by CD19+ cell numbers using FCM, demonstrating prolonged aplasia until the last follow-up

CP-48: Flow cytometry and Effusions in Lymphoproliferative process - Conjunction of Knowledge and Technique

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Department of Pathology, Government Medical College and Hospital, Nagpur

INTRODUCTION: Cytopathologists often face lymphocyte-rich effusions. Distinguishing between reactive and indolent lymphoma is challenging via microscopy alone. Flow cytometry is valuable for diagnosing lymphoma in body cavity fluids, whether for known diseases or new cases.

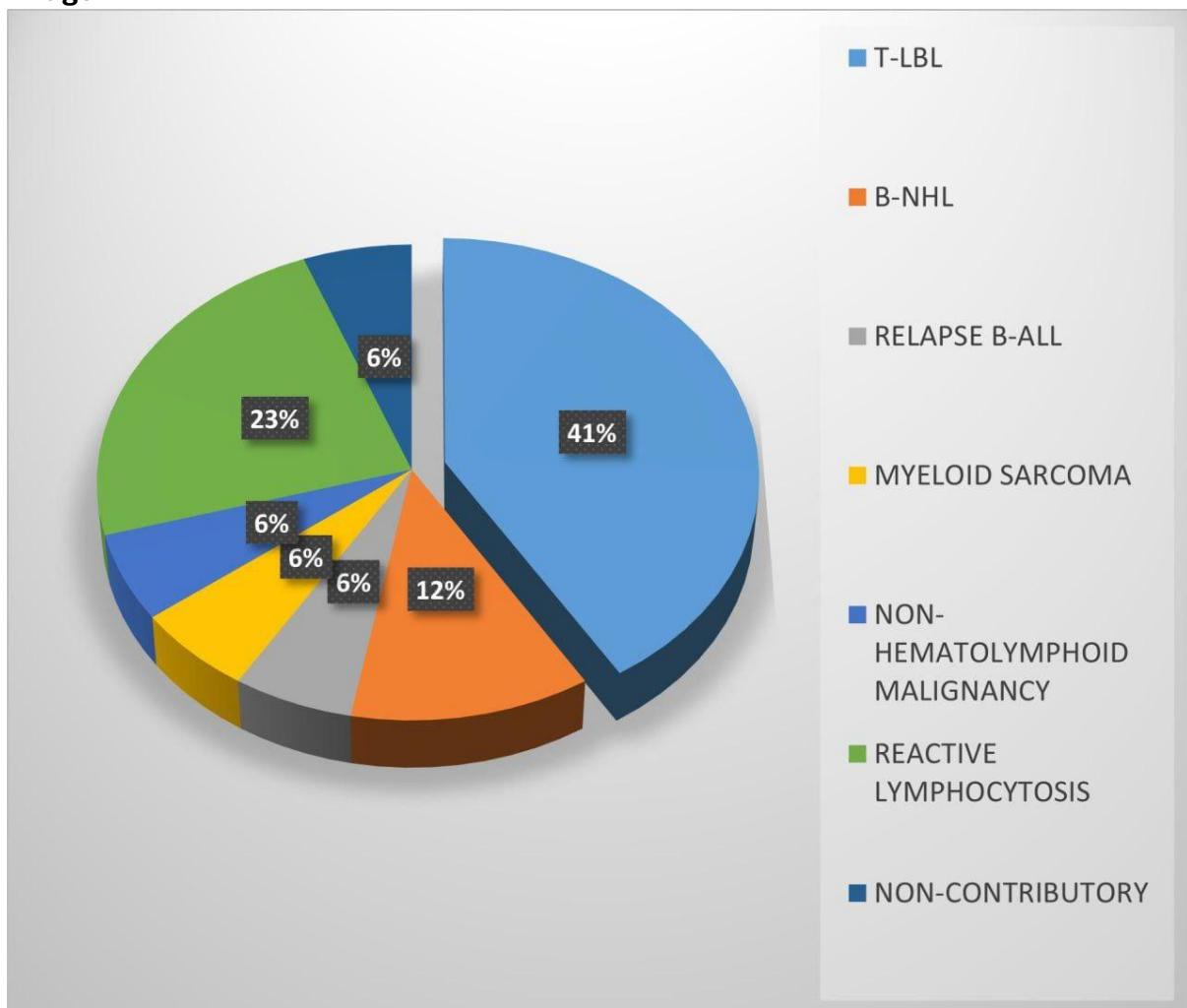
AIMS AND OBJECTIVE: To evaluate the utility of flow cytometric immunophenotyping (FCI) in effusion samples for diagnosis and subtyping of hematolymphoid malignancy.

MATERIAL AND METHODS: All morphologically suspected cases of hematolymphoid malignancy involving body fluids were included. The fluids included were pleural, pericardial, peritoneal and cerebrospinal fluid (CSF). The effusion samples underwent processing using the “lyse-stain-wash” method for staining surface markers, while the “lyse-stain-perm-stain-wash” protocol was employed for cytoplasmic markers. Single tube flow cytometry assay was done in hypocellular sample such as CSF. Appropriate antibody panels were applied and ran on 10 color and 3 laser Navious Beckman Coulter flow Cytometer. The LMDs were interpreted on Kaluza software. Cell blocks from fluid sediments were prepared for immunocytochemistry (ICC).

RESULTS: Out of the seventeen morphologically suspected cases of hematolymphoid malignancies, flow cytometric immunophenotyping show 7 cases of T cell lymphoblastic lymphoma, 1 case of B cell acute lymphoblastic leukemia, 1 case of Large B cell lymphoma, 1 case of Burkitt lymphoma, 1 case of Non hematolymphoid malignancy, 1 case of myeloid sarcoma, 4 cases of reactive lymphocytosis and 1 case being non-contributory.

CONCLUSION: Immunophenotyping through FCI has been highly beneficial in analyzing body cavity effusions. FCI is vital for distinguishing reactive lymphocytosis from lymphomas with puzzling morphology and very well complements IHC (Immunohistochemistry) and molecular techniques for accurate lymphoproliferative disorder diagnosis in fluids. It helps in distinguishing hematolymphoid malignancies from undifferentiated malignancies. It is particularly valuable in situations where a biopsy is life-threatening, as it allows for diagnosis through fluid analysis. Flow cytometry also aids in the prognostication of diseases and is instrumental in diagnosing extra-nodal lymphomas.

Image



CP-49: The best things in life are free, so is side scatter in flow cytometry!

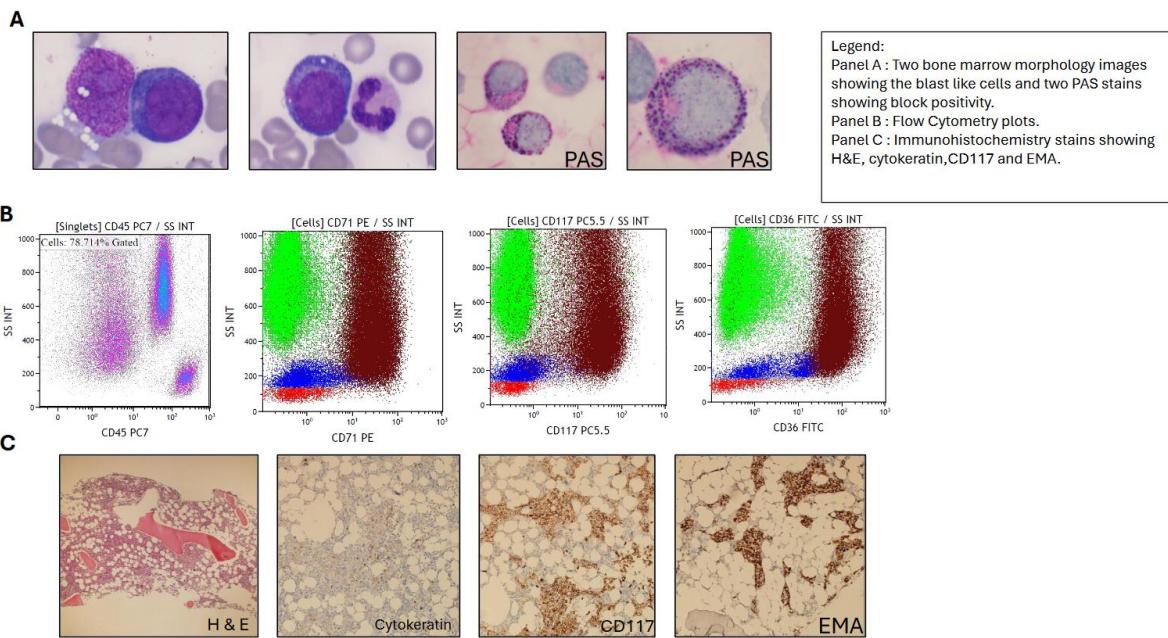
Mohammed Bilal Ali, Anwaar AlHathrami, Mohammad Burney, Badr AlZarooni, Rania Medhat, Hassan AlYassin, Zakaa Noori, Mouza AlSharhan, Pankhi Dutta

Dubai Health , Department of Laboratory Medicine & Pathology

Background: Flow cytometry is the most common method for diagnosing and classifying acute leukemias. A comprehensive panel of antibodies is used in most laboratories are not created with solid malignancies in mind. We wish to present a case of metastatic carcinoma of the bone marrow which was initially diagnosed as Acute Erythroid Leukemia.

Clinical Case: A 41-year-old woman of Asian descent, with history of essential thrombocytosis since 2014 and on 1.5gram hydroxyurea daily (stopped in view of pancytopenia) presented to the emergency. Initial investigations revealed pancytopenia (WBC count $1.6 \times 10^3/\mu\text{L}$, haemoglobin 6.2 g/dL, & platelet count $72 \times 10^3/\mu\text{L}$. Of other investigations, the Lactate Dehydrogenase level was significantly raised (15,268U/L). Peripheral blood smear was unremarkable. Bone marrow aspiration was performed to rule out transformation to acute leukemia / myelofibrosis. The aspiration showed sheets of large atypical immature appearing cells with basophilic cytoplasm, possibly erythroid blasts. PAS stain showed granular /block positivity. Flow cytometry showed a blast like population, dim positive to negative for CD45 and positive for CD36, CD71, CD117 and negative for CD235a. Other myeloid and lymphoid markers were negative. Based on the above, an initial diagnosis of acute erythroid leukemia was made. However, trephine biopsy immunohistochemistry showed these neoplastic cells to be positive for CD117, Pan cytokeratin, EMA, epithelial antigen and E-cadherin, while being negative for all other lineage specific markers. Glycophorin A and C were negative. Whole body CT scan did not reveal any mass. However, PET scan showed a heterogenous ill-defined mass near the pancreas. A final diagnosis of metastatic poorly differentiated/undifferentiated carcinoma of unknown primary was given. The patient died before definite treatment could be started. On reviewing again, the flow cytometry showed very unusually high scatter of the atypical cells. The scatter was the only finding that could have helped arriving at an earlier diagnosis as glycophorin (which is specific for erythroid leukemia) is positive in only a subset of cases.

Conclusion: Our case highlights the fact that along with antigen expression, the scatter properties of abnormal cells are also very important factors to be considered in the analysis of every case.



CP-50: Intercepting Acute Myeloid Leukemia with mutated NPM1 and FLT3 (ITD) by Next Generation Sequencing - A case report with deceptive morphology and immunophenotype mimicking Acute Promyelocytic Leukemia (APL)

Dr Sara Razzak, Dr Safia Mustafa, Dr Anuradha Sekaran, Dr Sohini Chattopadhyay

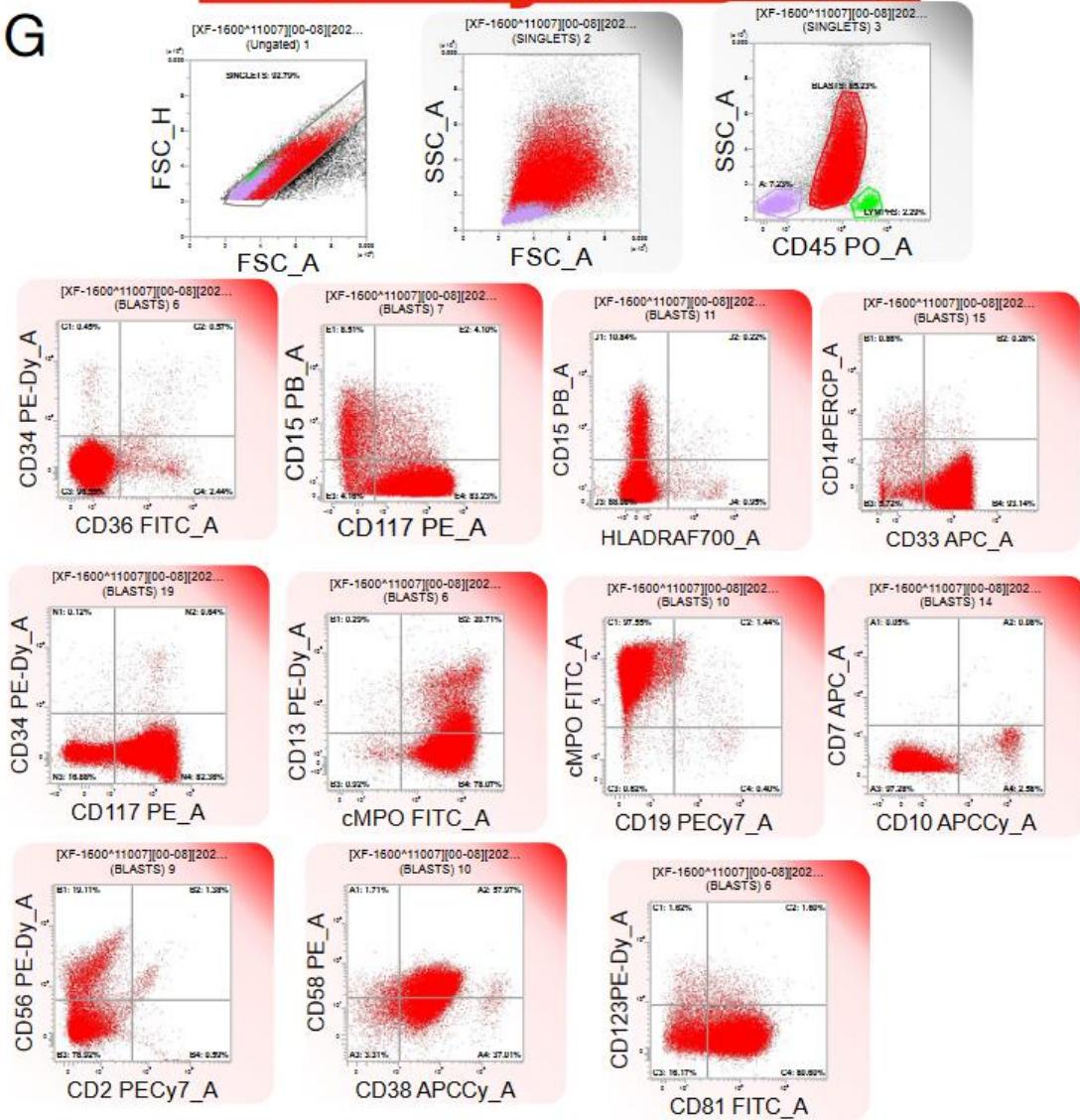
AIG Hospitals, Gachibowli, Hyderabad

Abstract

Here we present a case of Acute Myeloid Leukemia (AML) with mutated Nucleophosmin (NPM1) mutation and FLT3 internal tandem duplication (ITD) which mimics microgranular variant of Acute Promyelocytic Leukemia (APL) immunophenotypically and morphologically and role of Next Generation Sequencing (NGS) in such cases for definitive diagnosis. A 70 year old man post CABG presented with lower limb swelling pain and dyspnea. On evaluation, white blood cell count of 16,500/cumm, hemoglobin of 13.3 g/dl, a platelet count of 50,000/cumm with abnormal coagulation parameters. A peripheral smear revealed 65% blasts, demonstrating prominently cleaved bilobed nuclei with fine chromatin prominent nucleoli and occasional cup like nuclei. Flow cytometry revealed High side scatter and abnormal myeloid blast expressing myeloperoxidase, CD13, CD33, CD117, CD38 and lacking CD34, HLADR. Cytogenetics showed normal karyotype and negative for Promyelocytic Leukemia/Retinoic acid receptor a (PML-RARA) fusion by Fluorescence In Situ hybridization (FISH). Next Generation Sequencing (NGS) revealed pathogenic variant in Nucleophosmin 1 (NPM1), Isocitrate dehydrogenase (IDH2), FLT3 Internal Tandem Duplication (ITD), Serine/arginine-rich splicing factor 2 (SRSF2) and Neuroblastoma-RAS (NRAS) genes . AML with mutated NPM1 and FLT3 (ITD) often shows loss of CD34, HLADR with nuclear features mimicking Acute Promyelocytic Leukemia (APL), although some blast showing cup like nuclei. Hence we conclude that it is important to consider this entity AML with NPM1 and /or FLT3 (ITD) in cases that have features suggestive of Acute Promyelocytic leukaemia (APL) in absence of PML-RARA by FISH and importance of NGS in such cases for correct diagnosis, treatment and better prognosis of the patient.

Flow cytometry

G



16th Annual Conference & Workshops TCS 2024

POSTER ABSTRACTS (BASIC)

BP-01: Cytomorphological spectrum of breast lesion in ELDERLY FEMALE

Dr SUMANTA RAUTH, DR CHETNA JAIN

Jhalawar medical college, Rajasthan

Breast lumps are common complaints of women. Breast cancer is the leading cause of morbidity and mortality in women. The main goal of FNAC breast is to confirm a radiological and clinical benign lesion so as to avoid unnecessary surgery and also to confirm a malignant diagnosis to allow definitive treatment planning. The aim and objective were to study the cytomorphological spectrum of all breast lesion in elderly females >60 years and to determine the clinical characteristics of palpable breast lump. This is a prospective cross sectional study wherein FNAC findings were noted in breasts of 32 elderly females. The most common benign lesion found were granulomatous lesions (3 out of 10 benign breast lesions.) Duct carcinoma was overwhelmingly common - 20 out of 22 patients (90.9%). Breast lumps (left > right) were the most common symptom of breast lesions whereas breast tenderness was the most common sign in both benign and malignant breast. Consistency of breast lumps were mostly soft in benign cases and mostly hard in malignant cases. Upper outer quadrant locations were the most common for breast lesions. The most common duration of lesion was 0-15 days in benign lesions and was 3-6 months in malignant lesions. Benign breast lesions are almost always mobile (88%) whereas malignant breast lesions are almost always fixed (85%). Out of three granulomatous lesions, one belonged to Grade I BIRADS, two belonged to Grade II BIRADS. One each of fibroadenoma, benign breast and fibrocystic disease had BIRADS II. There was one patient of duct ectopia with BIRADS III and two benign proliferative breasts with BIRADS III. 20 patients of duct carcinoma had BIRADS V. Duct carcinoma showed Lymph node metastasis in 67% of cases. When their particular cytologic features are clear, benign breast tumors are typically easy to recognise. Despite FNAC's high degree of accuracy it has a few shortcomings. So in order to make a correct diagnosis it should be used in conjunction with radiographic analysis and complete clinical correlation.

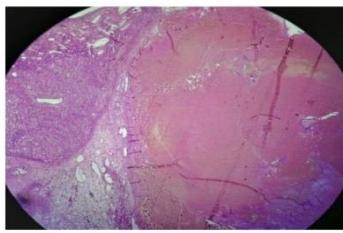
BP-02: Ectopic ovarian pregnancy
Dr SUMANTA RAUTH, DR CHETNA JAIN
Jhalawar medical college, Rajasthan

A 23 years old female came to gynae OPD with complaining of pain in lower abdomen, amenorrhea of 8 weeks. On examination, urine pregnancy test was positive. BP – 100/60 mm hg, pulse- 102/min. During emergency laparotomy, ruptured ovarian ectopic pregnancy was diagnosed and wedge resection of the ovary was only done. Histopathological examination confirmed it to be an ovarian ectopic pregnancy. Methodology: 1) Tissue Fixation by 10% neutral buffered formalin 2) Gross Examination 3) Tissue Processing by using graded ethyl alcohol, clearing by xylene and embedding with paraffin wax as agent 4) Tissue sectioning by microtome 5) Staining with Hematoxylin and Eosin for direct examination under microscope. Microscopic Features: Multiple Sections Examined from ovarian tissue shows corpus luteum with hemorrhage and cystic follicles. The ovary also show blood clot with numerous chorionic villi and trophoblastic tissue. Conclusion: IUD is one of contraceptive methods which prevents intra uterine implantation in 99.5% cases. If implantation occurs with IUD, it is tubal implantation in 95% of cases and it is very rare in other places such as ovary. The most important risk factor of ovarian ectopic pregnancy is IUD as in this study it was showed. Plenty of times its not possible to pinpoint the exact diagnosis at the time of surgery so its imperative to go for HPE in all such cases so as to negate any diagnostic confusion.

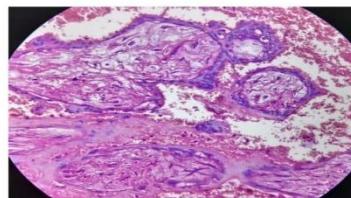
OVARIAN ECTOPIC PREGNANCY

Background: A 23 years old female came to gynae OPD with complaining of pain in lower abdomen, amenorrhea of 8 weeks. On examination, urine pregnancy test was positive. BP – 100/60 mm hg, pulse- 102/min. During emergency laparotomy, ruptured ovarian ectopic pregnancy was diagnosed and wedge resection of the ovary was only done. Histopathological examination confirmed it to be an ovarian ectopic pregnancy

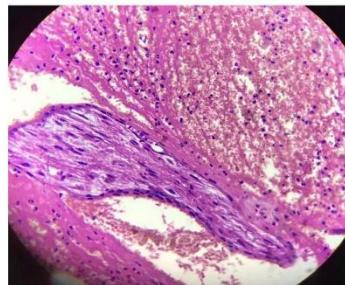
LOW POWER VIEW



HIGH POWER VIEW



HIGH POWER VIEW



Methodology: 1) Tissue Fixation by 10% neutral buffered formalin 2) Gross Examination 3) Tissue Processing by using graded ethyl alcohol, clearing by xylene and embedding with paraffin wax as agent 4) Tissue sectioning by microtome 5) Staining with Hematoxylin and Eosin for direct examination under microscope

Microscopic Features: Multiple Sections Examined from ovarian tissue shows corpus luteum with hemorrhage and cystic follicles. The ovary also show blood clot with numerous chorionic villi and trophoblastic tissue.

Conclusion: IUD is one of contraceptive methods which prevents intra uterine implantation in 99.5% cases. If implantation occurs with IUD, it is tubal implantation in 95% of cases and it is very rare in other places such as ovary. The most important risk factor of ovarian ectopic pregnancy is IUD as in this study it was showed. Plenty of times its not possible to pinpoint the exact diagnosis at the time of surgery so its imperative to go for HPE in all such cases so as to negate any diagnostic confusion.

BP-03: SuperNova dye SNv650 conjugates performance on different sample types for various immunological and cellular studies

Jasmine gouda Beckman coulter life sciences, Bangalore

Introduction: Beckman Coulter Life Sciences introduces SuperNova (SN) v650, the fourth addition to their SN dye family, which includes SNv428, SNv605, and SNv786. SNv650 has a maximum excitation of 414 nm and an emission maximum of 646 nm, detected using the 660/30 bandpass filters of a flow cytometer. SNv650 polymer dye conjugates are produced under manufacturing controls consistent with GMP. These dyes exhibit minimal non-specific staining due to their proprietary formulation, providing greater confidence in flow cytometry results. This study evaluates the performance of SNv650 dye conjugates in combination with other SuperNova dyes across different sample types and lysing conditions. The goal is to assess the compatibility of SNv650 conjugates with various workflows, supporting multiple immunological and cell-based research applications.

Methods: Three lots of SuperNova conjugates were produced and stained on whole blood specimens and were compared against commercially available polymer dye conjugates at their recommended doses. SNv650 conjugates were tested along with other SuperNova and classical dye conjugates in a multicolor panel to assess performance in both single-color and multicolour settings. Various workflows, fragile sample types, and processing reagents were tested with SNv650 conjugate-containing panels to evaluate the impact on performance. These studies were performed on a Beckman Coulter Life Sciences flow cytometer.

Results: Median fluorescence intensity, percentage recruitment of positive cells, stain index, and non-specific binding on negative populations were analyzed. All three lots of SuperNova conjugates showed significantly higher stain indices compared to commercial conjugates. SNv650 conjugates demonstrated equivalent performance in single-color and multicolor panels, including other fluorochromes like FITC, PE, etc., without affecting the characteristics of other markers. A distortion matrix was developed to guide users for compensation in multicolor panels. When SNv650 conjugates were tested on whole blood specimens using different Beckman Coulter Life Sciences lysing solutions, the lymphocyte purity was consistently over 75%, with no significant differences across protocols.

Conclusion: This study highlights the superior performance of SNv650 conjugates compared to other polymer dyes, showcasing their application in various workflows and panels. SuperNova conjugates offer enhanced brightness with minimal non-specific staining, improving the identification of low-intensity antigens and rare populations. With the addition of SNv650, we can now fill all 13 channels of the CytoFLEX flow cytometer, making a comprehensive range of reagents available for advanced flow cytometry applications.

BP 04: Platelet Inhibitory Effects of Phytochemicals: A flow Cytometry Study

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Department of Microbiology and Cell Biology, Indian Institute of Science, Bangalore-560012, India

Platelets are essential for haemostasis and thrombosis. However, increased platelet activation due to various pre-disposing factors can lead to thrombotic disorders like coronary artery diseases and stroke. Various phytochemicals have been well studied for their biological effects such as antiviral, anticancer, immunomodulatory, anti-inflammatory, antimicrobial, anti-diabetic, anti-parasitic, and anti-HIV properties etc and possess beneficial effects for human health. Therefore, we undertook a study to evaluate various phytochemicals for their effect of platelet activations. We stimulated platelet activation with various platelet agonists like collagen, Adenosine 5'-diphosphate (ADP), thrombin, thromboxane A₂ analogue U46619, calcium ionophore A23187 and Phorbol 12-myristate 13-acetate (PMA). The samples were analyzed by flow cytometer (BD FACS Canto™ II) to determine the level of platelet activation. The effects Epigallocatechin gallate (EGCG) and betulinic acid on various agonist induced platelet activation will be discussed.

BP 05: New automation-friendly, 96-well plate, ready-to-use 8-color dry reagent panel for immunophenotyping of human whole blood and PBMCs by flow cytometry

S. Mazumder, G. Edicheri Alinkeezhil, N. Dupas, R. Bowers, S. Saif, M. Kapinsky

Beckman Coulter Life Sciences

Introduction: Laboratory automation in flow cytometry attempts to reduce laboratory intensive manual and tedious pipetting steps which could further lead to human error. Automation-friendly 96-well plate flow cytometry solutions are ideal for pharmaceutical, academic, and CRO laboratories that perform testing on large numbers of samples and/or various test conditions. Our ready-to-use, pre-formulated dry antibody panel in 96-well plate format provides researchers all the antibodies within the wells of the plate which could provide walkway solution for sample processing.

Methods: EDTA-anticoagulated whole blood and peripheral blood mononuclear cells (PBMCs) were used to study immunophenotyping by using DURAClone IM Phenotyping BASIC plate, part# C97741 (2 x 96 tests + Single color compensation control plate, 8 single stains in duplicate). Samples were collected as per Ethical guidelines and processed within 96-well plate, RBC's were lysed with FLEXLyse (RUO) and acquired in CytoFLEX LX flow cytometer. 7AAD was used as in drop in reagent to evaluate live/dead cell. The studies were conducted in Beckman Coulter Life Sciences (Bangalore, India and Miami, USA). Our experiments were performed using automation on the Biomek i7 liquid handling automated workstation integrated with a CytoFLEX LX flow cytometer and microplate centrifuge demonstrating a fully automated flow cytometry workflow.

Results: Our study show flow cytometry data for both whole blood and PBMC samples stained with the first ready-to-use DURAClone IM Phenotyping BASIC plate (RUO) in a shallow 96-well plate containing a dry reagent antibody panel to identify major lymphocyte and monocyte subpopulations for the following markers: CD45, CD16, CD56, CD19, CD14, CD3, CD4 and CD8.

Conclusion: The DURAClone IM Phenotyping BASIC plate (RUO) is the first ready-to-use flow cytometry solution available in automation-friendly dry reagent 96-well plate format that is compatible with whole blood and PBMC samples for both no-wash and wash staining protocols.

BP 06: Evaluation of cellular wound healing using Flowcytometry

Reshma Murali and Anugya Bhatt

Division of Thrombosis Research, Department of Applied Biology, Biomedical Technology Wing, Sree Chitra Tirunal Institute for Medical Sciences and Technology, Trivandrum, India

Wound healing is a well-orchestrated complex cellular process and it involves the spatial and temporal synchronization of different cell types with unique roles in the phases of hemostasis, inflammation, proliferation, and Extra cellular matrix remodeling. Flowcytometry is one of the well-advanced single cell technologies that is utilized to uncover phenotypic and functional heterogeneity within a complex process like wound healing. Platelets and fibroblasts are the major cell types involved in wound healing process. In the present study, we focused to analyse the effect of MSCs secretome and activated platelet rich plasma on impaired wound healing in-vitro models using flow cytometry. Adipose tissue derived MSCs (ADMSCs) and PRP was isolated from healthy rabbits (Newzeland White, 2kg, 6 months) after obtaining Institutional Animal Ethics Committee clearance (SCT/IAEC-439/July/2022/113). Impaired wound healing studies were done in hypoxia and hyperglycemia induced cellular models. Hypoxia was induced by incubating fibroblasts in 600µm H₂O₂ for 2hrs. Cytoprotective effect of MSC secretome on hypoxia induced fibroblasts were evaluated by apoptosis assay and confirmed by Live/dead assay. The effect of MSC secretome on macrophage polarization was evaluated using M1 and M2 macrophage surface markers CD163 and CD80 by flowcytometry. Clinical relevance of hyperglycemia on platelet activation was studied using P selectin marker. We observed that MSC secretome treatment significantly increased live cells in live/dead assay and induced macrophage polarization. Our results showed that hyperglycemia induces hyperactivation in platelets that may lead to prolonged inflammatory phase in diabetic patients. Further in vivo evaluation of MSc secretome on impaired wound healing need to be performed in diabetic models as future prospectives.

BP 07: Exploring the burden of Paroxysmal nocturnal hemoglobinuria in western Rajasthan: A tertiary care center study.

DEVANSH OJHA, Dr. Nagarjun Sai, Dr. Abhishek Purohit, Dr. Poonam Abhay

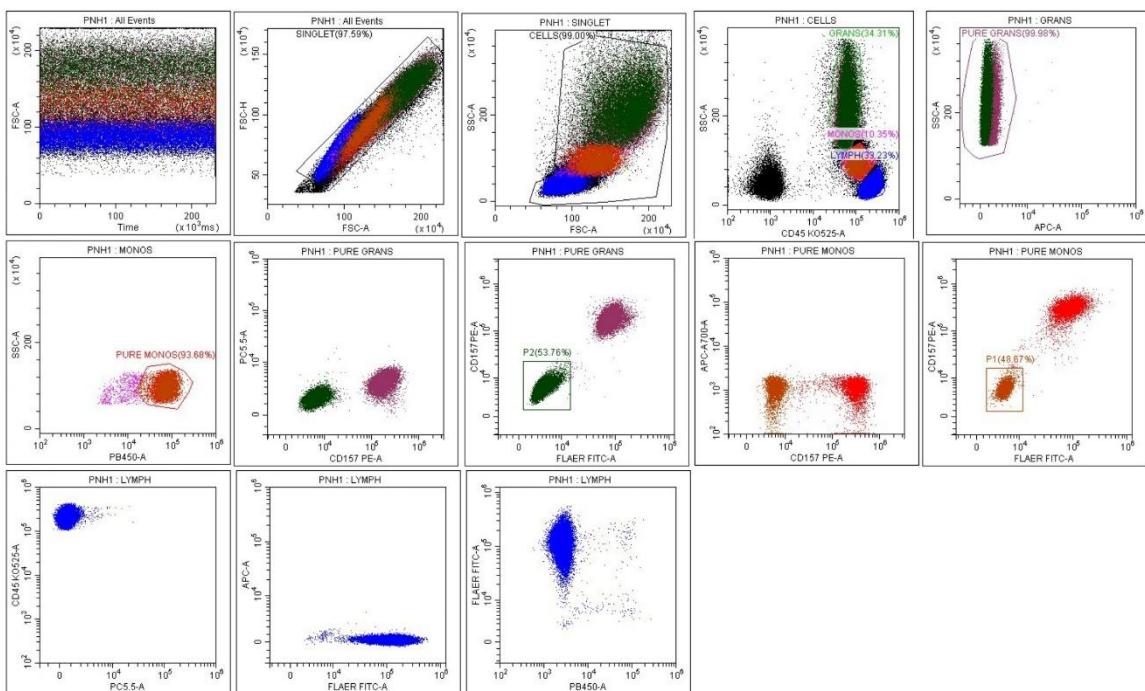
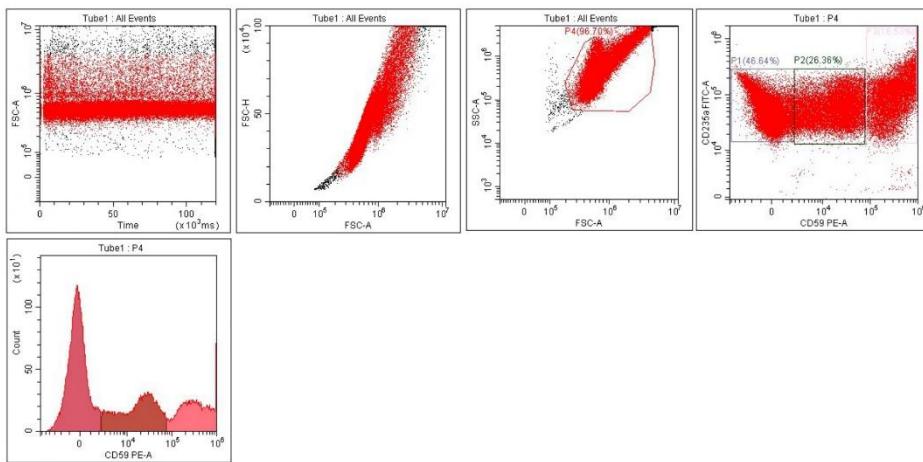
Department of Pathology & Lab Medicine, AIIMS Jodhpur, Rajasthan

Background: Paroxysmal nocturnal hemoglobinuria (PNH) is a rare, acquired clonal hematopoietic stem cell disease that presents with hemolytic anemia, bone marrow failure, and thrombosis. It originates from a multipotent hematopoietic stem cell that acquires a mutation in the phosphatidylinositol glycan anchor biosynthesis class A (PIG-A) gene, located on chromosome Xp22.1. This mutation results in a partial or complete deficiency of all glycosylphosphatidylinositol-anchored proteins (GPI-AP). Deficiencies in proteins such as CD55 (decay-accelerating factor, DAF) and CD59 (membrane inhibitor of reactive lysis, MIRL) leading to complement-mediated cell lysis and other clinical manifestations. Flow cytometry is the gold standard test for detecting PNH clones.

Methods: This cross-sectional study was conducted at AIIMS, Jodhpur over a period of three years. Laboratory and clinical details were extracted from the hospital information system. Suspected PNH cases were evaluated using flow cytometry with a comprehensive immuno-phenotyping panel, including CD24 (PC5.5), CD157 (PE), CD14 (PC700), CD64 (PB), CD45 (KRO), and FLAER (FITC). Samples were acquired using the Beckman Coulter DxFLEX instrument, and data analysis was performed with CytExpert software.

Results: A total of 135 suspected cases of PNH were included in the study, comprising 75 males and 60 females, with ages ranging from 4 to 75 years. Of these, 7 cases (prevalence – 5.18%) were diagnosed with PNH based on the double-negative expression of FLAER and CD157 on granulocytes and monocytes, along with negative expression of CD235a on RBCs. PNH clone detection and clone size estimation were performed for all diagnosed cases.

Conclusion: Flow cytometry is currently the preferred laboratory technique for providing an accurate, sensitive, standardized, and widely applicable diagnosis of PNH. This study helps in exploring the cases of PNH and estimation of its burden in a tertiary care center in western Rajasthan using flow cytometry.



BP 08: Detection of Myeloid Signature in COVID-19 Patients Using Flow Cytometry in Response to SARS-CoV-2 Infection

Mahesh Kumar M1, Vijayalakshmi V2, Bindu Sadanandan2, Neha Girish1, Shankar Patabhiraman1

1. Beckman Coulter Life Sciences, Bangalore, India

2. Department of Biotechnology, MS Ramaiah Institute of Technology, Bangalore, India

Introduction: Myeloid cells, such as dendritic cells and monocytes, play a key role in SARS-CoV-2 Infection as early indicators by secreting the cytokines against viral infection. In this study, a standardized dry antibody panel, a cocktail of liquid antibodies was used to identify myeloid cells in COVID-19 patients. The recruitment of myeloid dendritic cells such as CD11c+CD16+ and CD11c+CD1c+, as well as a subset of monocytes such as CD14+CD16- classical monocytes, CD14++CD16+ intermediate monocytes and CD16+CD14- non-classical monocytes, CD169+HLA-DR+ and CD64+ HLA-DR+ monocytes was evaluated.

Methods: Whole blood samples with EDTA anticoagulant from healthy control (n=45) and from patients with RT-PCR-confirmed SARS-CoV-2 infection (n=33 for 3 consecutive days) were taken for antibody staining. Whole blood samples were stained with the above panels, followed by RBC lysis, PBS wash and resuspension of cells in Fixation Buffer. Cells were acquired using a CytoFLEX flow cytometer. Expression of the markers of myeloid dendritic cells and subset of monocytes and CD169+HLA-DR+ and CD64+ HLA-DR+ monocytes were reported as the percentage of positive cells for respective receptors.

Results: There was significant upregulation of CD169+HLA-DR+ (p value <0.0001) and CD64+HLA-DR+ (p value <0.0001) monocytes in COVID-19 patients in comparison with the healthy control samples. CD11c+CD16+ myeloid cells were significantly increased in patients (p value <0.0001) as compared to healthy controls. No changes were observed for subset of monocytes in comparison to healthy controls. There was no expression of CD64 on granulocytes in patient samples.

Conclusions: The increased CD169+HLA-DR+ and CD64+HLA-DR+ monocytes indicates that monocytes were activated during SARS-CoV-2 infection and resulted in induction of a severe form of COVID-19. The increased CD11c+CD16+ myeloid dendritic cells indicate early response of dendritic cells against SARS-CoV-2. These observations help in understanding myeloid immune response during early stage of COVID-19 infection and identifying targets for developing treatment strategies.

BP 09: Nimbolide inhibits the growth of Miltefosine resistant Leishmania donovani parasite - A Flow Cytometry based in-vitro study

Radheshyam Maurya and Madhulika Namdeo

Department of Animal Biology, School of Life Sciences, University of Hyderabad, India

Visceral leishmaniasis (VL) is a disease transmitted by vectors and caused by the parasitic protozoan *Leishmania donovani*. VL is a very severe manifestation of leishmaniasis and may be lethal if not treated early. The leishmania parasite developed resistance to most of the drugs used for its treatment, including Miltefosine (MIL). Therefore, it is crucial to identify novel antileishmanial compounds to prevent the development of MIL-resistant *L. donovani* parasites.

The Neem (*Azadirachta indica*) leaf extracts containing several bioactive molecules have shown antileishmanial activities. Among those compounds, nimbolide (NB) has been shown to possess both antibacterial and anticancer properties. In the present study, we investigate the antileishmanial properties of NB against MIL-resistant and MIL-sensitive *L. donovani*. The MTT experiment and growth curve analysis demonstrate that NB has a dose-dependent inhibitory effect on promastigote parasite. A scanning electron microscopy (SEM) study has shown that NB treatment significantly influenced the size and shape of promastigote parasites.

A flow cytometry study reveals that treatment with NB modified the activities of mitochondrial superoxide generation (ROS), mitochondrial membrane potential (MMP), cell cycle, and cell proliferation. Treatment with NB decreased the efficiency of intracellular amastigote infection and proliferations, which was further confirmed by a decrease in IL-10 cytokine production in human macrophages infected with *L. donovani* (THP-1 cells). Thus, studies reveal that Nimbolide has the potential to be a very effective therapeutic candidate for treating Leishmaniasis and controlling the development of MIL-resistant parasites

BP 10: Consistent satisfactory performance of Cyflow users in national CD4 proficiency testing program conducted in India

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Introduction: Although viral load testing plays a primary role in making therapeutic decision in the management of people living with HIV (PLHIV), importance of CD4 lymphocyte count estimation cannot be undermined as it plays crucial role in monitoring of disease progression and initiation of prophylactic therapies. ICMR-NITVAR conducts proficiency program for ensuring quality and accuracy of the CD4 estimation carried out under national AIDS and STD control programme covering the entire geographic area of India. This study was conducted to assess the trend of performance observed across commonly used CD4 testing machines in proficiency testing (PT) rounds over past 2 years.

Methodology: ICMR-NITVAR runs PT program for CD4 testing Laboratories in India with more than 450 public and private laboratories enrolled in it. PT rounds are conducted twice in a year in which two indigenously stabilized blood samples are distributed to the participating laboratories. Algorithm A using robust analysis was used to analyse PT data. Z scores were used to assess the performance and the score of with +/-2 was considered to be satisfactory.

Results: Two commonly used CD4 machines in the program include Cyflow and PIMA. Satisfactory performance was reported by more than 90% of Cyflow participants in past four rounds while 54.54% to 82% of PIMA participants showed satisfactory performance. Coefficient of variation of results reported by Cyflow users varied from 2.98 to 8.44% while that for PIMA users was more than 20%. More than 92% FACS Presto users participated in the last two PT round also showed satisfactory performance with CV varying from 2.78 to 6.64%.

Conclusion: Trend monitoring of results reported by CD4 proficiency testing participants indicated consistent performance of cyflow as well as FACS Presto users while higher unsatisfactory performances by PIMA users indicated a need for further investigating reasons for the failures.

Image

	Total no. of instruments	QC 55		Total no. of instruments	QC 55		Total no. of instruments	QC 56		Total no. of instruments	QC 56	
ROUND NQA 25		ABS			% ABS			ABS			%	
INSTRUMENT		SATIS	UNS		SATIS	UNS		SATIS	UNS		SATIS	UNS
CYFLOW	94	93(98.93)	1(1.06)	89	85(95.50)	4(4.49)	94	93(98.93)	1(1.06)	89	84(94.38)	5(5.61)
PIMA	56	46(82.14)	10(17.85)				51	40(78.43)	11(21.56)			

	Total no. of instruments	QC 57		Total no. of instruments	QC 57		Total no. of instruments	QC 58		Total no. of instruments	QC 58	
ROUND NQA 26		ABS			% ABS			ABS			%	
INSTRUMENT		ABS	SATIS	UNS	%	SATIS	UNS	SATIS	UNS	SATIS	UNS	
CYFLOW	121	119(98.34)	2(1.65)	95	85(89.47)	10(10.52)	121	119(98.34)	2(1.65)	95	90(94.73)	5(5.26)
PIMA	47	28(59.57)	19(40.42)				47	32(68.08)	15(31.98)			

	Total no. of instruments	QC 59		Total no. of instruments	QC 59		Total no. of instruments	QC 60		Total no. of instruments	QC 60	
ROUND NQA 27		ABS			% ABS			ABS			%	
INSTRUMENT		ABS	SATIS	UNS	%	SATIS	UNS	SATIS	UNS	SATIS	UNS	
CYFLOW	112	111(99.10)	1(0.89)	89	87(97.75)	2(2.24)	112	111(99.10)	1(0.89)	92	84(91.30)	8(8.69)
PIMA	44	27(61.36)	17(38.63)				44	28(63.63)	16(36.36)			
FACSPRESTO	13	13(100)	0	13	12(92.30)	1(7.69)	13	13(100)	0	13	12(92.30)	1(7.69)

	Total no. of instruments	QC 61		Total no. of instruments	QC 61		Total no. of instruments	QC 62		Total no. of instruments	QC 62	
ROUND NQA 28		ABS			% ABS			ABS			%	
INSTRUMENT		ABS	SATIS	UNS	%	SATIS	UNS	SATIS	UNS	SATIS	UNS	
CYFLOW	193	185(95.85)	8(4.14)	182	166(91.20)	16(8.79)	193	188(97.40)	5(2.55)	182	171(93.95)	11(6.04)
PIMA	45	28(62.22)	17(37.77)				44	24(54.54)	20(45.45)			
FACSPRESTO	43	43(100)	0	41	41(100)	0	43	42(97.67)	1(2.32)	41	41(100)	0

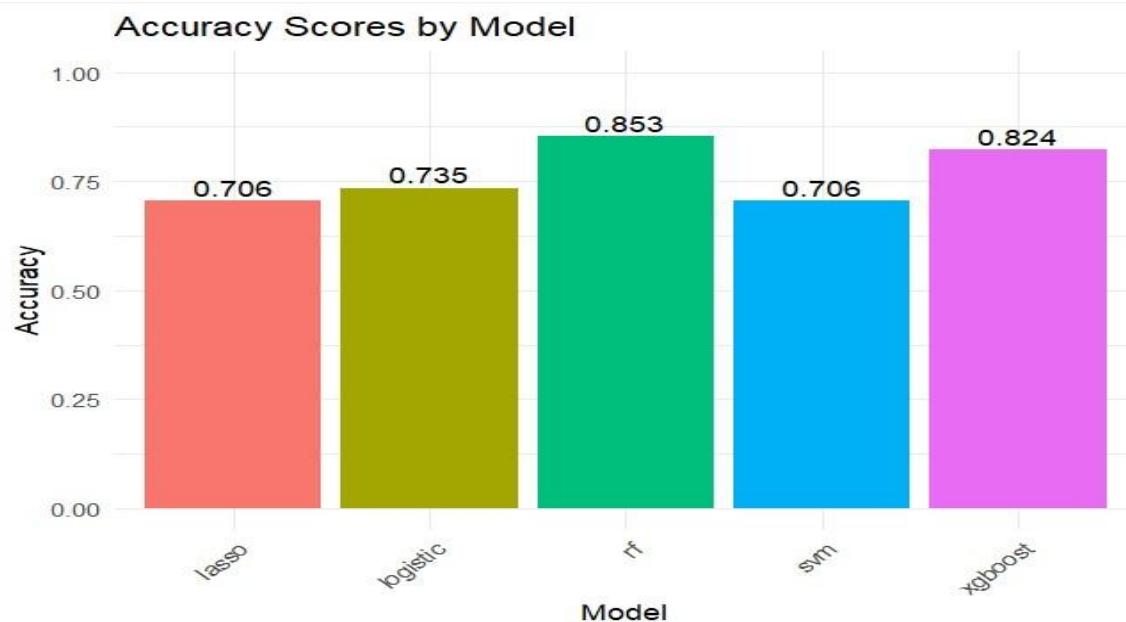
BP 11: Common Variable Immunodeficiency Disorder: A Decade of Insights from a Cohort of 150 Patients in India and the Use of Machine Learning Algorithms to Predict Severity

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Common Variable Immunodeficiency (CVID) is a heterogeneous disorder characterized by impaired antibody production and recurrent infections. This study investigated the clinical and immunological features of CVID in 150 Indian patients over a decade at a tertiary care center. The median age of diagnosis was 18 years, with a male predominance (62%). Most patients (66.6%) presented with a severe phenotype, with recurrent respiratory tract infections being the most common clinical manifestation (84.2%). Gastrointestinal complications and autoimmune manifestations were observed in 45% and 21% of patients, respectively. All patients exhibited hypogammaglobulinemia, with varying IgA and IgM levels. B-cell analysis revealed reduced class-switched memory B cells in 64.4% of patients. Nine adult patients presented with late-onset combined immunodeficiency. Genetic testing, performed on 52 patients, identified underlying monogenic causes in 29 pediatric and 15 adult patients, with LRBA deficiency being the most common genetic defect. We also developed a novel machine learning-based severity prediction model for CVID patients, utilizing lymphocyte subsets, class-switched memory B cell counts, and serum immunoglobulin levels. Random Forest outperformed other models, achieving an accuracy of 0.853 (95% CI: 0.840-0.866). Feature importance analysis identified Th-Tc ratio, CD19, and IgM levels as the most influential predictors for severity prediction. This study highlights the diverse clinical and immunological features of CVID in Indian patients, emphasizing the need for early diagnosis and individualized management strategies. The severity prediction model based on commonly available immune parameters may help in directing treatment strategies to improve patient outcomes. These findings contribute to a better understanding of CVID in the Indian population and offer a potential tool for clinical decision-making.

Image



BP 12: CD8 T cell responses against SARS-CoV-2 Spike antigen differ qualitatively from the responses against antigens from common viral infections.

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Dr Ashwini Shete, 1Shubhangi Bichare, 1Sheetal Mulay, 1Dr Madhuri Thakar, 2Sulakshana Bansode, 2Nidhi Thakur, 2Dr Shashikant Dudhgaonkar, 2Dr Preeti Chavan-Gautam, 2Dr Girish Tillu.

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Introduction:

CD8+ T cells are major arm of antiviral immune response. These cytotoxic T cells are able to clear viral infection by eliminating infected cells. They secrete cytokines like interleukin, IFN- γ and TNF- α to exert cytotoxic response in order to clear pathogen. CD107a is degranulation marker present on activated CD8+ T cells. This study serves as qualitative comparison between SARS-CoV-2 Spike antigen and CEF peptides derived from human Cytomegalovirus, Epstein-Barr Virus and Influenza Virus which is antigen for common viral infection.

Methodology:

Healthy male participants (n=24) from age 20 to 30 were enrolled and 10 ml of venous blood was collected in EDTA vacutainers from the participants. Peripheral blood mononuclear cells were separated and stimulated overnight with SARS-CoV-2 Spike antigen and CEF peptide. PMA/Ionomycin was used as positive control. The next day, intracellular cytokine staining assay was performed and the cells were acquired on multicolor flow cytometer (FACSAria Fusion). Monofunctional (CD107a, IFN- γ , IL-2 & TNF- α) and polyfunctional (by Boolean gating) T cells responses were analyzed using FlowJo software. Wilcoxon matched paired t test was performed using GraphPad Prism5 for statistical analysis.

Results:

Frequency of CD107a expressing CD8+T cells was significantly higher in response to Spike protein than that against CEF peptides ($p = 0.0208$) indicating higher degranulation inducing capacity of SARS-CoV-2 Spike antigen. Frequency of IFN- γ expressing CD8+T cells was significantly higher in response CEF than against Spike protein ($p=0.0407$). Similarly, bifunctional and polyfunctional (expression of 3 or more cytokines) CD8+T cell responses were higher with CEF than that against the Spike protein ($p=0.0405$ -one tailed and <0.0001 , respectively).

Conclusion: Significant differences were observed in CD8+ T cells responses against CEF antigen and SARS-CoV-2 Spike antigen. Polyfunctional response was higher in response to CEF antigen along with IFN- γ expression but CD107a response was higher in response to the Spike protein.

BP 13: The Clinicopathologic and Immunophenotyping study of Chronic Myelogenous Leukemia Blast Phase (CML-BP) :A Retrospective Analysis of 65 Patients from A Tertiary Care Hospital.

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Background:CML is consistently associated with an acquired genetic abnormality,the Philadelphia chromosome (Ph1). It evolves from a chronic phase into terminal phase of acute phase(blast crisis).The role of flow cytometry in CP-CML is unknown,which is reserved for the determination of the blast lineage in the blast phase (BP-CML).It is important to distinguish myeloid vs lymphoid blast crisis, since the latter often responds to chemotherapy the former is largely resistant to treatment. **Aims & Objectives:** This the aim to study was done to evaluate the immunophenotyping characteristics of CML-BP.

Materials &Methods: It's a retrospective study from tertiary care hospital between 2017 to Till date.Data of the subjects in CML blast crisis was compiled and analyzed for the immunophenotypic categorization of the blast population.The relevant clinical,flow cytometry ,bone marrow ,molecular diagnostics and other relevant laboratory data were retrieved from hospital and departmental medical records.The samples were processed by 10 colour flow cytometry using Stain-Lyse-Wash method.Extramedullary blast proliferation case samples were from fluid cytology specimens and retrieved from FFPE embedded tissue archives;and IHC performed wherever necessary. **Inclusion criteria:** All cases were diagnosed as CM -BP by latest WHO guidelines .Presence of blast proliferation in fluid cytology specimens or other non hematopoietic locations included. **Exclusion criteria:** Cases with CML-CP,cases with insufficient data were excluded.

Results : A total of 65 cases retrieved. Age range from 16-78(mean age 44) years. M:F:1.6:1,with male preponderance.Blast phase evolved from chronic phase in 54 patients and denovo detected in 11cases.In 50 cases both bone marrow and peripheral blood tests done,15 patients had blasts in extramedullary sites proliferation.Splenomegaly in 26, hepatomegaly in 8 cases respectively. On immunophenotyping analysis 76% myeloblastic,18% were lymphoblastic,5 % undifferentiated and, 1% mixed phenotype type respectively.Among 50 myeloblastic cases 8 cases had monocytic differentiation. Among 15 cases of extramedullary blast proliferation,CSF and lymph node were the most common sites.

Conclusion : Flow Cytometry is the diagnostic investigation mode for CML-BP cases.It helps in characterizing the blast lineage because treatment different for myeloid and lymphoid blast phase.

BP 14: Possible role of Chaetocin in reversing of Prelamin A accumulation and HIV latency in HIV infected individuals

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Background: Prelamin A, a precursor of lamin A, gets accumulated in response to oxidative stress and has been implicated in premature and physiological aging. Accumulated Prelamin A induces DNA damage causing genomic instability and premature senescence. Since HIV infection is characterized by chronic oxidative stress, we assessed if HIV infected cases have higher Prelamin A levels as compared to HIV uninfected controls. We also investigated effect of histone methyltransferase inhibitor on Prelamin A and P24 expression in the cases as depleting Suv39h1 results in improved DNA repair mechanisms and HIV latency reversal.

Methods: The study was conducted in asymptomatic virally suppressed HIV infected patients on ART for more than 5 years [n=70, M:F=36:34] and HIV uninfected controls [n=68, M:F=31:37] in the age-group of 40-55 years. Blood samples were collected for assessing levels of Prelamin A accumulation in CD4 and CD8 T cells by flow cytometry. PBMCs of the study participants were treated with Chaetocin (400nM), a histone methyltransferase inhibitor, to assess its effect on Prelamin A accumulation and intracellular P24 expression in HIV infected individuals. Prelamin A levels were analysed for their correlation with immunosenescent markers.

Results:

Higher median fluorescence intensity of Prelamin A expression was observed in CD4 and CD8+T cells ($p<0.0001$) in HIV cases as compared to control samples. Prelamin A accumulation in CD4+T cells correlated significantly with the frequency of CD57+CD4+T cells ($r=0.237, p=0.038$) as well as CMV IgG index ($r=0.229, p=0.049$). Prelamin A accumulation in CD4+T cells diminished significantly after treating them with Chaetocin (0.0273). P24 expressing CD4+T cells increased significantly after Chaetocin treatment ($p=0.0294$).

Conclusion: Prelamin A accumulation, a marker of DNA damage, might be used for detecting premature aging in HIV individuals. Possible role of histone methylation modulators in reversing premature aging as well as HIV latency needs to be explored further.

BP 15: Fabrication and characterization of plasma-treated electrospun membranes for blood filtration application

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Membrane-based leukodepletion filters reduce many clinically adverse effects during blood transfusions such as non-hemolytic febrile transfusion reactions, and platelet refractoriness. Polyethylene co-vinyl alcohol (EVAL) is a hemocompatible polymer proven for its leukodepletion efficiency. However, due to its hydrophobic character, it finds some limitations during blood filtration. In this current study, the wettability of the EVAL membranes were improved by blending it with a hydrophilic polymer such as polyvinyl pyrrolidone (PVP) and its plasma surface treatment. Contact angle measurements showed that incorporating PVP improved the wettability of the membrane. Blood filtration studies conducted with the modified membranes showed that the filtration time through the membranes was significantly improved after plasma treatment. The percentage of WBC adhesion, RBC recovery, platelet adhesion and percentage of hemolysis were studied. The residual leukocyte count after filtration was studied using flow cytometry. The flowcytometry data provides the percentage of leukodepletion of EVAL/PVP and plasma treated EVAL/PVP as 100 and $99.9 \pm 0.14\%$ respectively. The study thus shows that suitability of plasma treated electrospun EVAL/PVP membranes as a potential candidate for leukodepletion filter media.

BP 16: FAMILY-BASED STUDY TO EXAMINE THE ASSOCIATION BETWEEN GUT MICROBIOME AND TYPE 1 DIABETES MELLITUS.

Kritika Bakshi, Nisha Kapoor

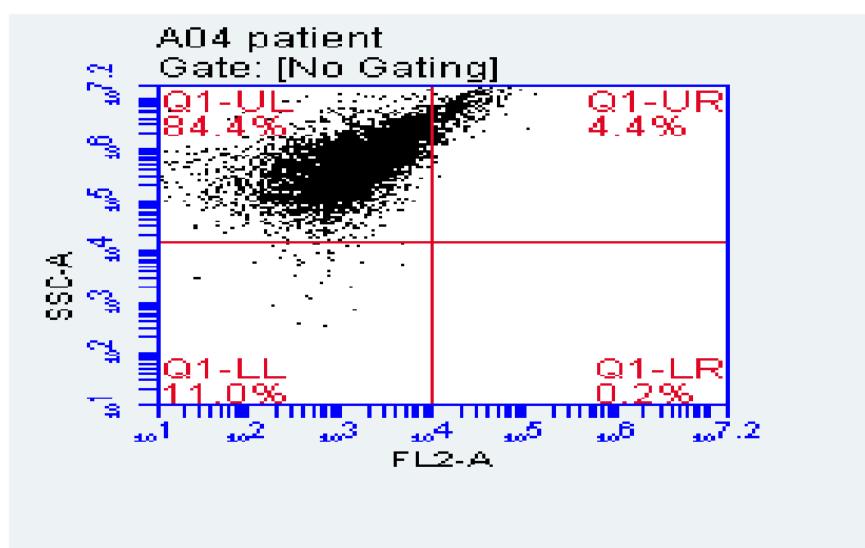
University of Jammu

Background: Gut dysbiosis and Type 1 diabetes mellitus (T1DM) are interconnected conditions that disrupt metabolic and immune functions. T1DM results from the immune system attacking insulin-producing β -cells in the pancreas, impairing blood sugar regulation. Simultaneously, gut dysbiosis, characterized by an imbalance in the gut microbiota, can increase intestinal permeability and trigger immune responses, exacerbating inflammation. This may worsen the autoimmune destruction of β -cells in T1DM patients, leading to poor blood sugar control. A healthy gut microbiome plays a crucial role in maintaining the gut barrier and producing short-chain fatty acids (SCFAs), which help regulate immune responses and glucose metabolism. Dysbiosis impairs these functions, contributing to insulin resistance and complications in T1DM.

Aim: The study aims to identify if there is an association between gut microbiome and T1DM, at family level. **Methodology:** In this study, the gut microbiome of a family was analysed, including a T1DM patient, the patient's mother, and the daughter. Faecal samples were collected, and culture-dependent methods were used to identify media that support the growth of gut microbes, based on previous research. After culturing, morphological and biochemical analyses were performed to identify the microbes. Flow cytometry was also conducted to assess microbial load.

Results: The study revealed that only a small fraction of the gut microbiota is culturable under aerobic conditions, with blood brain infusion agar effectively culturing fastidious bacteria. Flow cytometry showed a higher number of dead cells in the patient's gut, indicating a predominance of anaerobic microbes. The daughter of the patient had mostly simple, non-marker-expressing cells (61.6%), whereas the samples of patient and his mother were dominated by complex cells. Across all samples, the marker-expressing cell population remained small, ranging from 3.2% to 4.4%.

Conclusion: This study highlights the complex relationship between gut microbiota, T1DM, and immune system function. **Keywords:** Type 1 Diabetes Mellitus (T1DM), β -cells, Microbiome, Gut dysbiosis, SCFAs.



BP 17: Association between Innate Immune Modulation and Gut Microbiome Dynamics in Early Pregnancy

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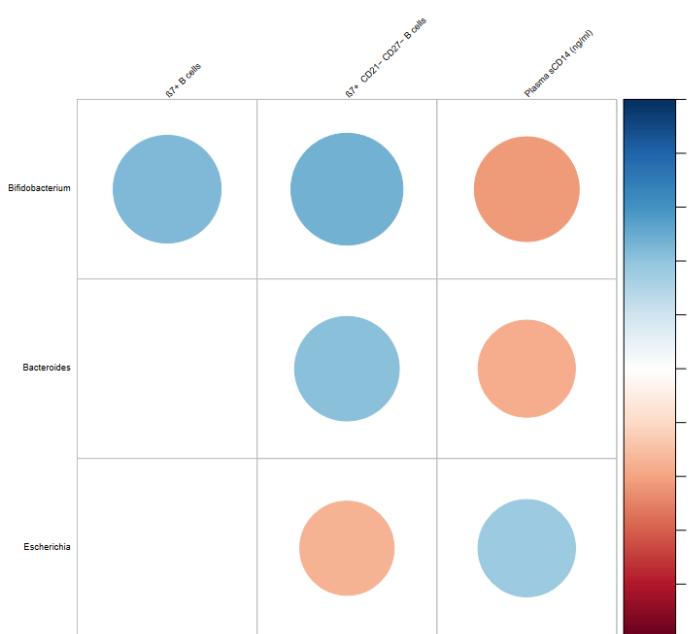
Early pregnancy induces profound immune changes essential for maternal and fetal health. Dysregulation of these responses can lead to adverse outcomes such as miscarriage and recurrent pregnancy loss. Since the gut microbiome has been suggested to modulate immune responses in both health and disease, we aimed to explore the link between changes in the gut microbiome composition with the immune modulation observed during early pregnancy. We recruited first trimester primigravida women along with non-pregnant controls. Whole blood immunophenotyping was conducted using BD FACSaria Fusion, and gut microbiome was assessed by 16S rRNA sequencing using Oxford Nanopore Technology (ONT). We observed a significant increase in granulocytic (CD14- CD15+) and monocytic (CD14+ CD15-) myeloid derived suppressor cells (MDSCs) in pregnant women, with a reduction in NKT-like cells (CD3+ CD56+). Cytotoxic NK cells (CD56dim CD16+) increased, while regulatory NK cells (CD56bright CD16dim) decreased, revealing a dynamic but balanced immune response. No significant changes were observed in T cell subsets when compared to non-pregnant women. Gut microbiome analysis revealed an increase in Bacteroidetes, which positively correlated with regulatory NK cells, promoting an anti-inflammatory environment. Conversely, increase in Proteobacteria and Actinobacteria correlated with pro-inflammatory immune cells (cytotoxic NK and NKT-like cells). To validate the findings related to observed changes in frequency of innate immune cells during pregnancy, we exposed THP1 monocytic cells to fecal supernatants of pregnant women, which exhibited increased expression of both pro-inflammatory cytokines (TNF- α , IL-6) and anti-inflammatory markers (TGF- β), compared to non-pregnant fecal supernatant. SCFA analysis revealed elevated acetate and propionate levels in pregnant women, supporting the role of gut microbiota in maintaining immune balance via these mediators, which activate genes in immunological pathways, as reflected in THP1 data. These findings suggest the possible role of gut microbiome in immune regulation during pregnancy, promoting maternal-fetal tolerance and successful pregnancy outcomes.

BP 18: Altered gut microbiome composition correlates with reduced frequency of integrin $\alpha 4\beta 7+$ B cells and increased immune activation in HIV+ individuals

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The intricate relationship between the gut microbiome, immune cells, and clinical outcomes is pivotal in understanding the pathogenesis of HIV. Detection of integrin $\alpha 4\beta 7$ on circulating B cells provides an estimate of various activated and resting B cell subsets trafficking between the systemic circulation and the gut. This study investigated the correlations between specific bacterial taxa, gut-homing B cell subsets, and clinical markers in HIV-infected individuals. We performed a cross-sectional study on antiretroviral therapy (ART)-naïve HIV+ (n=20) and HIV- (n=19) individuals recruited from ICTC and ART centres in Mumbai. Whole blood immunophenotyping was performed, and stained cells were acquired using BD FACSaria Fusion. Data were analyzed using FlowJoVX. Gut microbiome was analyzed by 16S rRNA sequencing of the V3-V4 region. Clinical parameters were measured in plasma and stool samples by ELISA. We observed a reduction in the frequency of gut-homing B cells in HIV+ ($70.5\% \pm 11.55$) individuals compared to HIV- ($81.9\% \pm 9.32$), indicating impaired mucosal immunity. Decreased relative abundance of *Bifidobacterium* and *Bacteroides* in HIV+ individuals ($0.56\% \pm 2.68$ and $<0.001\% \pm 0.24$) correlated positively with integrin $\beta 7+$ B cells and their subsets, while negatively correlating with plasma sCD14 levels ($1.07 \text{ ng/ml} \pm 0.4$), a marker for immune activation. These bacterial taxa may play a protective role in HIV pathogenesis by promoting the gut homing of integrin $\beta 7+$ B cells. Conversely, enrichment of *Escherichia* in HIV+ ($0.52\% \pm 4.16$) positively correlated with an increase in sCD14 and negatively with gut-homing B cell subsets. The loss of these cells could weaken the immune system's ability to control HIV replication at mucosal sites and respond to other pathogens, leading to increased morbidity. These findings highlight the importance of preserving mucosal immunity in HIV treatment and suggest that strategies aimed at supporting integrin $\beta 7+$ B cells may help mitigate immune dysregulation associated with HIV.



BP 19: Exploring the role of self-RNA-mediated activation of RIG-I-like receptors (RLRs) in the etiology of early-onset Type 1 Diabetes.

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Background : Self-RNA activates RIG-1-like receptors (RLR) in autoimmune diseases. In early type 1 diabetes (T1D), abnormal RLR expression may increase type-1 interferons, influencing disease susceptibility and progression. Aim The study aims to understand whether self-RNA-mediated RLR activation contributes to the pathogenesis of T1D.

Methodology: Plasmacytoid dendritic cells (pDCs) and monocytes were isolated from the peripheral blood of 25 T1D subjects and 20 healthy controls (HC). The cells were treated with RLR-agonist poly I:C, and phosphorylation of IRF3 (pIRF3) and IRF7 (pIRF7), key mediators of RLR activation, was measured using flow cytometry. The relative expression of RLR genes (DDX58 and IFIH1) was analyzed by qRT-PCR. To identify variants in RLR genes, Whole exome sequencing (WES) was performed in 70 T1D and 20 HC subjects. The uptake of self RNA-LL37 complexes, and IFN- α expression were assessed via flow cytometry. Molecular docking analysis was performed to identify potential antagonists of RLR.

Results : The baseline expression of pIRF3 was similar in T1D vs HC subjects. The expression of pIRF3 was enhanced post-poly I:C stimulation in both pDCs & monocytes in T1D ($33.74 \pm 2.37\%$ & $23.75 \pm 3.72\%$ respectively) compared to HC subjects ($23.18 \pm 1.79\%$ & $15.41 \pm 2.59\%$ respectively). RLR genes were observed to be upregulated in the T1D group. WES analysis showed heterozygous variants at exon 15(C>T; A946T) & exon 13 (C>T, H843R) of IFIH1 gene in 82.85% & 84.28% T1D subjects, respectively. Incubation with self RNA-LL37 complexes resulted in higher uptake of the RNA-LL37 complex (88.09% & 28.24 respectively) as compared to RNA alone (22% & 18% respectively). PDCs & monocytes stimulated with RNA-LL37 had higher pIRF3 ($19.50 \pm 0.52\%$ & $17.07 \pm 0.66\%$ respectively), pIRF7 ($30.9 \pm 0.45\%$ & $66.15 \pm 0.39\%$ respectively) & IFN- α expression ($7.45 \pm 1.45\%$ & $7.43 \pm 1.26\%$ respectively) expression compared to self RNA alone. Two RLR antagonists (DB09487 & DB01249) that could bind to RLR in an allosteric fashion, were identified by molecular docking analysis.

Conclusions: Besides, genetic variations in RLR genes, self RNA-LL37 complexes boost the expression of RLR and type-1 IFN signalling, suggestive of their role in T1D. Therefore, RLR antagonists present an alternate approach to inhibit T1D pathogenesis.

BP 20: Deciphering immune cell signature of Peripheral blood and Tumor Infiltrating Lymphocytes in NSCLC

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Advanced Centre for Treatment, Research and Education in Cancer (ACTREC) Tata Memorial Centre

Background: Immune cell composition in the tumor microenvironment critically influences disease progression and response to Immunotherapy such as immune checkpoint-inhibitors in NSCLC. Clinical practice relies on core biopsy to analyze immune signatures. However, biopsies are invasive, expensive, and often fail to capture tumor-immune heterogeneity. A noninvasive method to assess immune signatures in NSCLC is needed. In this study, we aimed to compare immune profiles between peripheral blood (PB) and tumor tissue, and determine if these profiles provide insights into tumor-microenvironment (TM).

Material & Methods Immune cell profiling in PB and tumor samples from 35 NSCLC patients was performed using a 16-color antibody panel (Table-1a). The cells were prepared using bulk lyse-stain method. Tumor tissue was first dissociated to isolate single cell suspension using BD Medi Machine II. Samples were acquired on BD LSRFortessa and post-acquisition analysis performed using Kaluza software.

Result CD8+T: Significant differences were observed between CD8+T cell subsets in PB and tumor tissue. Tumors had higher levels of CD8+Treg/EM/Tc1, whereas naïve/TE/CM/Tc2 cells were prevalent in PB. PD-1/TIM-3 were elevated on EM/TE cells in tumors, with TIM-3 also high on naïve cells. OX40 and TIGIT were prominent on naïve/CM in PB, OX40L was elevated in tumor EM (Table-1b). CD4+T: CD4+T cell subsets varied significantly, with Tregs/EM/Th1 cells Prevalent in tumors, while naïve/CM/Th2 cells were higher in PB. PD-1/PD-L1/CTLA-4/TIM-3/OX40/TIGIT varied across subsets, with PD-1 elevated on EM cells in tumors, and PD-L1 and CTLA-4 higher on naïve/CM cells in PB. TIM-3/OX40/TIGIT were enhanced on EM cells in tumors, with TIGIT elevated in blood CM (Table-1b).

Conclusion: PB shows differences in immune checkpoint expression and cell subsets compared to tumor tissue. While it reflects some patterns, it does not fully represent TM, especially for markers like PD-1 and TIM-3. Thus, PB alone is not a complete surrogate for tumor tissue.

BP 21: An approach to suppress autoimmune stem-like CD8+ T cells in Type-1 Diabetes using regulatory T cell-derived exosomes loaded with immune checkpoint ligands

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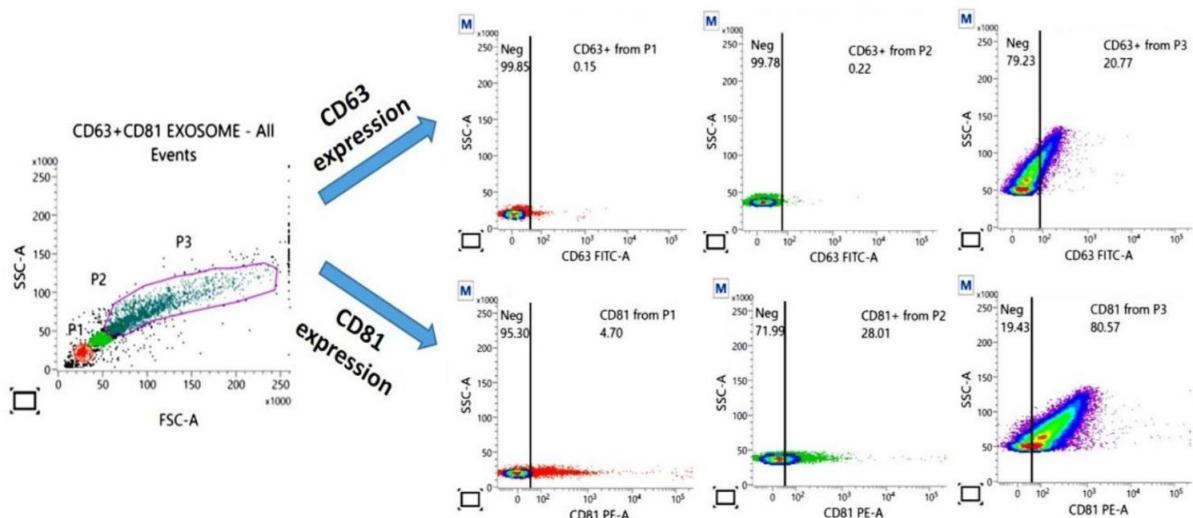
Background: Stem-like CD8+ T cells with self-renewal properties and effector functions hinder immunotherapy in type 1 diabetes (T1D). Aims: To inhibit β cell-specific stem-like CD8+ T cells by targeting immune checkpoint molecules (ICMs) utilizing agonist ligands loaded in exosomes derived from preproinsulin (PPI)-specific Tregs.

Methodology: Exosomes were isolated from peripheral regulatory T cells (Tregs) of T1D subjects and enriched using PPI-derived peptides. The isolated exosomes were characterized and ligands targeting the most frequently expressed ICMs (PD-1, TIGIT, BTLA) on peripheral CD8+ T cells were labeled and loaded in exosomes via sonication. These ligand-loaded exosomes (Exo-L) were then incorporated into autologous Tregs. Exo-L and Exo-L-infused Tregs were co-cultured with autologous PPI-pulsed effector CD8+ T (Teff) cells to evaluate their immunosuppressive abilities. Subsequently, single-cell RNA sequencing libraries were prepared using the BD Rhapsody Single-Cell Analysis System to profile the expression of ICMs on stem-like CD8+ T cells and choosing agonist ligands. The exosomes, loaded with these ligands, will be utilized to assess their impact on stem-like CD8+ T cells.

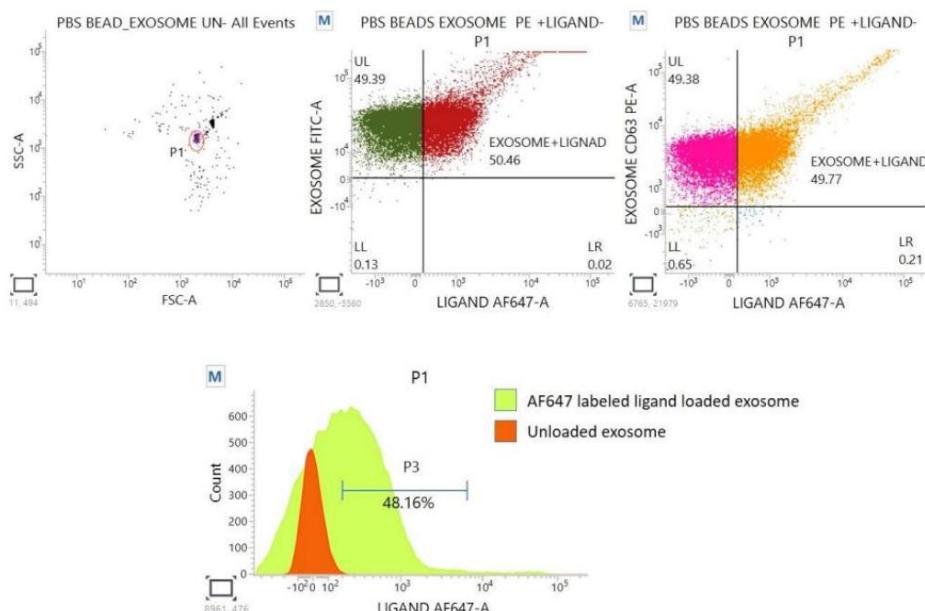
Results: Exosomes from PPI-Tregs exhibited typical spherical morphology and size (~100-150 nm), with integrity confirmed by TEM and ζ -potential analysis (ζ potential: -14.07 \pm 3.55 mV). These exosomes expressed specific tetraspanin markers (CD63, CD81, CD9), as demonstrated by flow cytometry and western blotting. Exosome internalization in Tregs was confirmed by confocal microscopy. Immunosuppression assays indicated a 30 \pm 0.47% (mean \pm SD) suppression of CD8+ Teff cells by Exo-L. Exo-L-treated Tregs showed significant downregulation of perforin, granzyme B, and IFN- γ in PPI-pulsed CD8+ T cells. These Tregs were also found to reduce CD8+ T cell mediated β -cell apoptosis in vitro as confirmed by annexin V-PI staining. Libraries have been sent for sequencing.

Conclusion: PPI-Treg-derived exosomes loaded with ICM-agonist ligands effectively suppress β cell-specific CD8+ T cell responses and have the potential to inhibit stem-like CD8+ T cells in T1D.

Characterization of exosomes-Flow cytometry

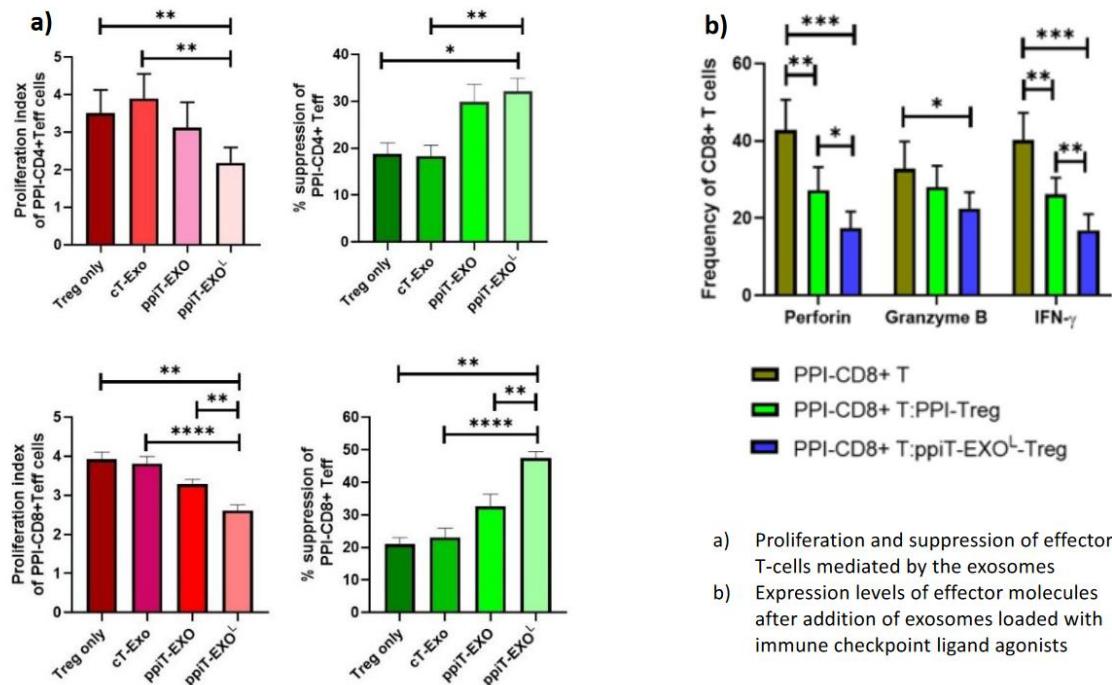


Flow cytometric detection of CD63 and CD81 positive exosome population in our ppIT-EXO preparation.
[P1= monomeric beads, P2= Intermediate cluster, P3= bead aggregates; exosomes are seen in all three gates]



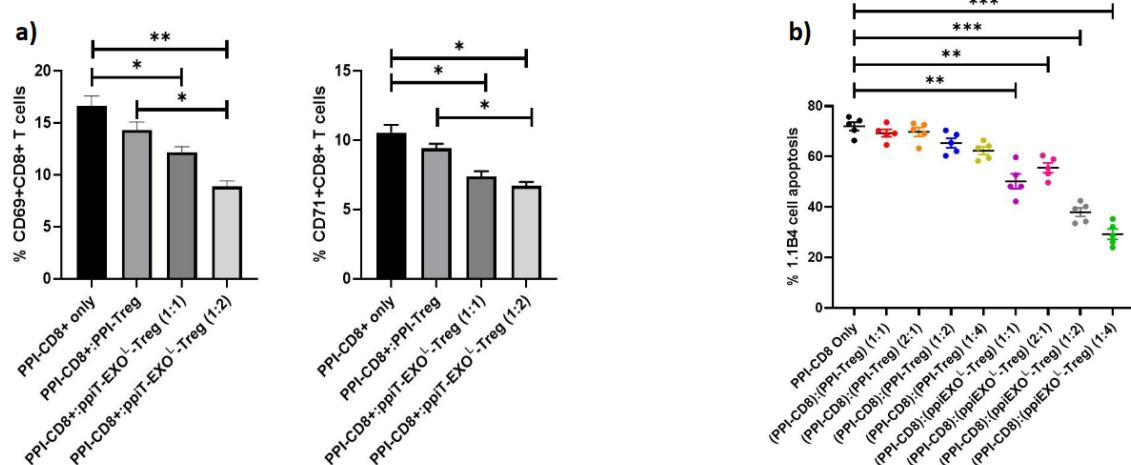
Dot plots showing the ligand containing exosome population stained with CFSE (acquired in FITC channel) and PE-anti CD63. B) Histogram showing the ligand positive exosome population. [P1= monomeric beads]

Determination of tolerogenic potential of the engineered exosomes



- a) Proliferation and suppression of effector T-cells mediated by the exosomes
b) Expression levels of effector molecules after addition of exosomes loaded with immune checkpoint ligand agonists

Determination of tolerogenic potential of the engineered exosomes



- a) Expression levels of activation markers after addition of exosomes loaded with immune checkpoint ligand agonists
b) Protection of 1.1B4 (β cell) from PPI-CD8+ T cell mediated apoptosis by ppIT-EXO^L-Tregs

BP 22: Antibody repertoire diversity correlates with neutralization breadth in COVID-19 convalescent individuals

Suprit Deshpande, Mohammed Yousuf Ansara Jyoti Sutar,f Payel Das, Nitin Hingankar, Sohini Mukherjee, Priyanka Jayal,a Savita Singh, Sreevatsan Raghavan, Supriya Chauhan Shweta Shrivastava,Chaman Prasad, Sangeeta Chauhan, Bhabatosh Das, Gaurav Batra, Guruprasad Medigeshi, Devin Sok Shinjini Bhatnagar,Pramod Kumar Garg,Jayanta Bhattacharya

Identifying features of development of an effective antigen-specific B cell repertoire could aid in understanding of the infection and/or vaccine driven affinity maturation of B cells and the resulting humoral immune responses. In the present study, through Fluorescence activated cell sorting followed by sequencing, antigen specific B cell repertoire was correlated with neutralization breadth of the monoclonal antibodies isolated from a COVID-19 convalescent individual (C-03-0020). Briefly, spike receptor binding domain specific B cells were sorted from PBMCs, followed by isolation, cloning, sequencing and generation of dual antigen-positive IgG as complete antibodies. We confirmed their binding to the receptor binding domain by ELISA and then observed their neutralization profile against the circulating/evolving SARS-CoV-2 variants through in vitro neutralization assays. Two novel neutralizing monoclonal antibodies (MAbs), THSC20.HVTR11 and THSC20.HVTR55, representing unique B cell lineages, were isolated from an unvaccinated donor C-03-0020 and compared to five Mabs previously reported from the same individual with distinct epitope specificities and B cell lineages. THSC20.HVTR11 was found to neutralize Omicron BA.1 and BA.2 potently while THSC20.HVTR55 neutralizes most of the pre-Omicron wave variants but fails to neutralize all Omicron variants.

In conclusion, antibodies isolated from C-03-0020 donor who was infected with ancestral SARS-CoV-2 in early 2020 were found to be varied in terms of both epitope specificities, B cell lineages of origin as well as their capability to neutralize all known variants of concern. Establishment of unique lineages leading to diverse B cell repertoire immediately following infection may have contributed to the broad polyclonal humoral immune response.

BP 23: Immunophenotypic Clues to Cytogenetic and Molecular Abnormalities

Dr. Seemitr Verma, Dr. Sindhura Lakshmi**

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Manipal Academy of Higher Education, Manipal, Karnataka, India*

Background: This study investigates the cytogenetic and molecular profiles of pediatric patients diagnosed with B-cell Acute Lymphoblastic Leukemia (B-ALL) and Acute Myeloid Leukemia (AML), aiming to identify immunophenotypic clues related to underlying genetic abnormalities. The research was conducted from January 2022 to December 2023.

Methods: A total of 28 cases of Acute Lymphoblastic Leukaemia (ALL) were analyzed for Fluorescence In Situ Hybridization (FISH) and ploidy analysis, while 2 cases of ALL were evaluated using Next-Generation Sequencing (NGS). Additionally, 7 cases of AML were studied for FISH and ploidy analysis, and 4 cases underwent AML NGS.

Results: The B-ALL cohort demonstrated significant hyperdiploidy, with high hyperdiploidy noted in cases featuring gains in chromosomes 4, 10, and 17. The most frequent aberrations were trisomies of chromosomes 9, 10, and 21. Molecular analysis revealed the absence of common translocations such as BCR::ABL1 and ETV6::RUNX1 in most cases, highlighting the genetic heterogeneity within this subgroup. In the AML cohort, notable findings included multiple instances of t(8;21), with associated mutations in PTPN11, IDH1, and NPM1 linked to varying prognosis. Hyperdiploidy and specific mutations were associated with distinct immunophenotypic markers, suggesting potential prognostic implications.

Conclusion: The findings indicate a complex interplay between immunophenotypic characteristics and genetic abnormalities in paediatric B-ALL and AML. Recognizing these immunophenotypic clues may enhance diagnostic precision and inform treatment strategies, underlining the necessity for integrated approaches in paediatric haematological malignancies. I would suggest doing more research to explore the prognostic significance of these correlations.

Table 1: Pearson Correlation of immunophenotypic markers between HLADR positive and negative AML

	HLA DR -	HLA DR +	P value
CD117	18	20	0.592
CD64	24	23	0.613
CD36	4	9	0.226
CD14	9	11	0.713
CD15	14	18	0.178
CD34	8	23	0.0003
CD7	2	9	0.043
CD19	0	3	0.238
CD56	10	10	0.64

BP 24: Analysis of select immune parameters and cytokine profile to define occurrence of 'Honeymoon Phase' in type 1 diabetes.

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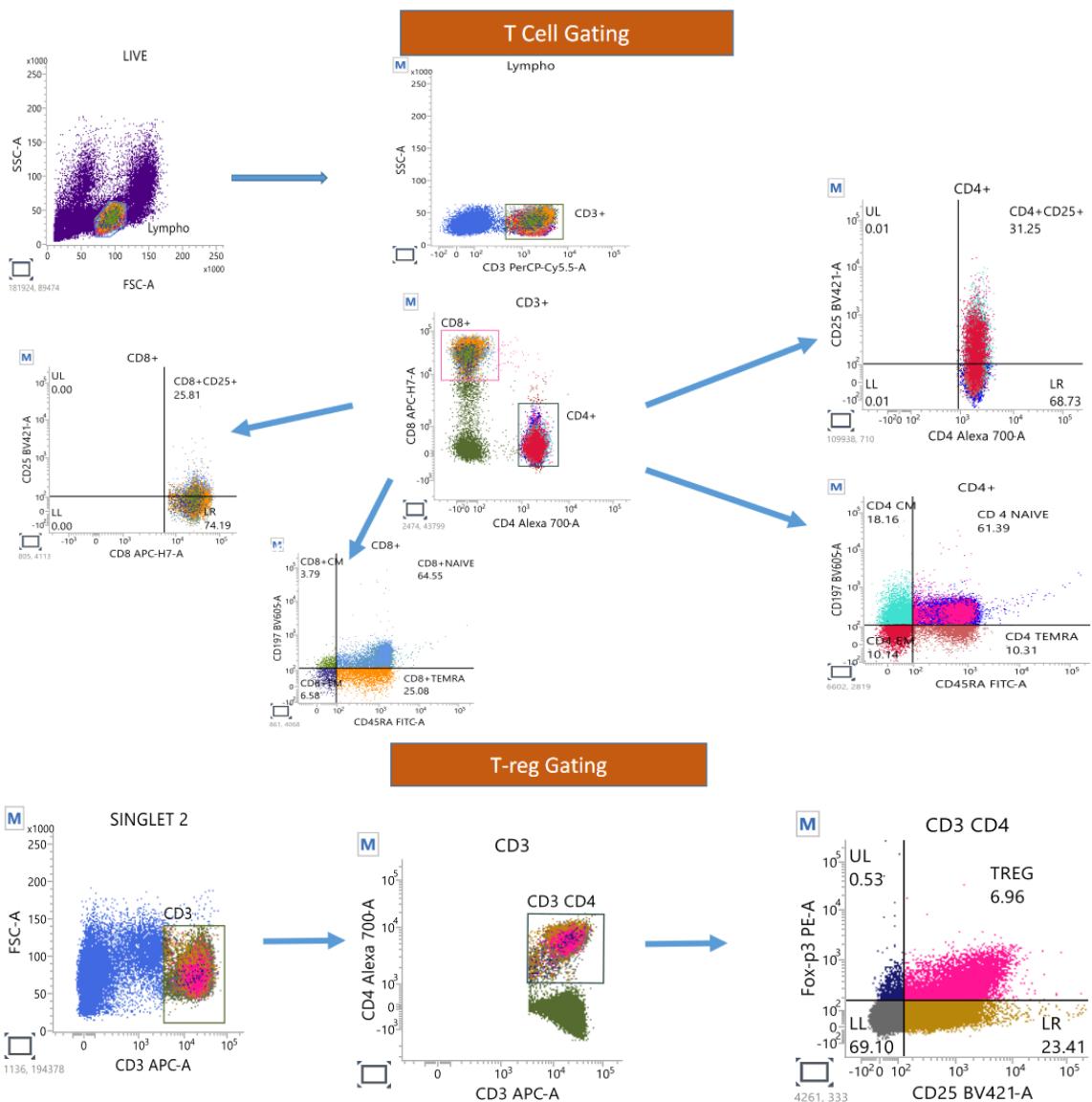
2Department of Translational and Regenerative Medicine, Post Graduate Institute of Medical Education and Research (PGIMER), Chandigarh, India

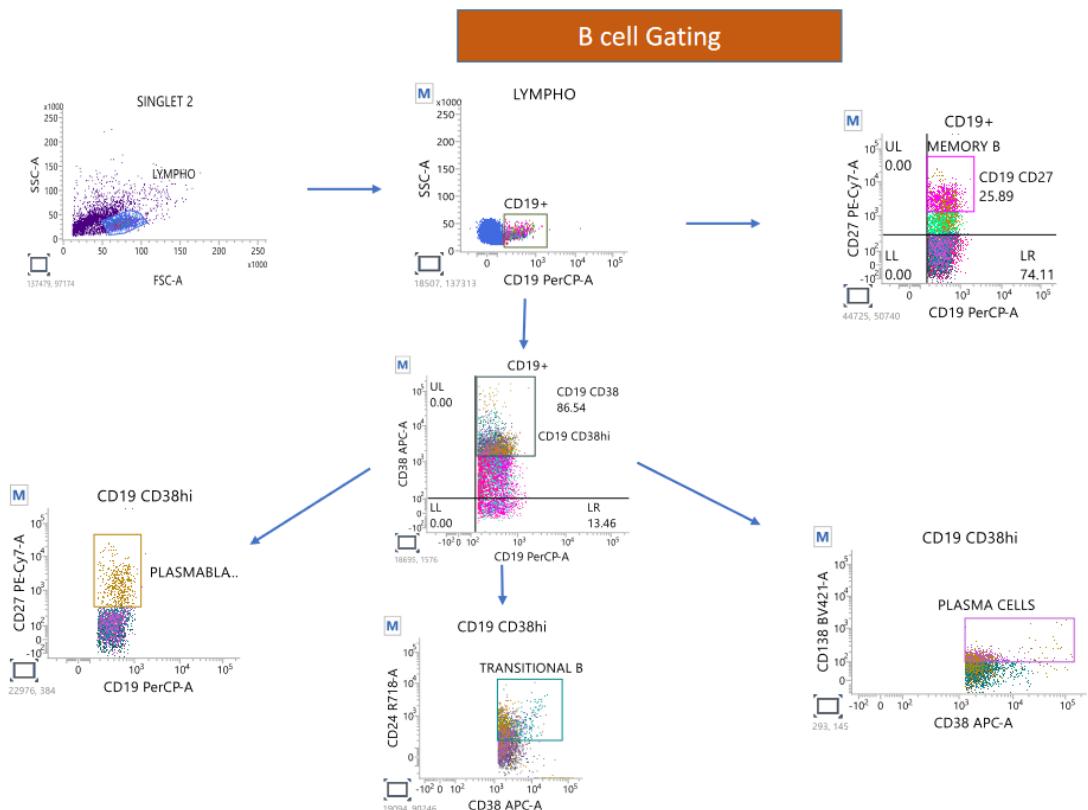
Background: Around 30% of T1D (Type 1 diabetes) patients experience a "honeymoon period" with reduced exogenous insulin requirement, though its genetic and immune markers are unclear. Aim: To characterize the genetic, serological, and immunological parameters of T1D patients in the honeymoon phase and in the non-honeymoon phase.

Methodology: A total of 20 T1DHP (Honeymoon phase) and 30 T1DNHP (Non Honeymoon phase) were recruited. PBMCs were isolated, stained with T and B cell surface antibodies, and analyzed via flow cytometry. Serum samples were tested for MIP-1 α , MCP-1, and serological markers (GAD-65, IAA, IA-2). HLA class I and II typing was performed to identify antigen specific T cells.

Results: Significant heterogeneity in HLA phenotypes was observed between the T1DNHP and T1DHP groups. Specifically, frequencies of HLA A26:01 (18.42% vs. 3%), DRB103:01 (45.71% vs. 19%), and DQB1*02:01 (47.08% vs. 18.07%) differed markedly, while other HLA alleles showed similar frequencies. Autoantibody-positivity was higher in T1DNHP at 90% compared to 76% in T1DHP. Median serum cytokines MIP-1 α and MCP-1 were elevated in T1DNHP (0.86 pg/ml vs. 0.20 pg/ml for MIP-1 α ; 44.92 pg/ml vs. 23.05 pg/ml for MCP-1), with T1DHP levels rising after exiting that period (1.93 pg/ml and 40.45 pg/ml, respectively). EM, CM and TEMRA CD4 T cells were high in T1DHP than T1D NHP (18.54% vs 10.47 %), (27.49 vs 19.46) and (4.795% vs 1.96 %) respectively along with its CD8 populations (28.75 % vs 15.70%), (5.7% vs 2.67%) and (34.02 vs 28.83)%respectively. T1DHP also exhibited a higher percentage of Tregs (7.9% vs. 6.02%). B cell subsets, including memory, transitional, and plasma cells, were lower in T1DHP than in T1DNHP.

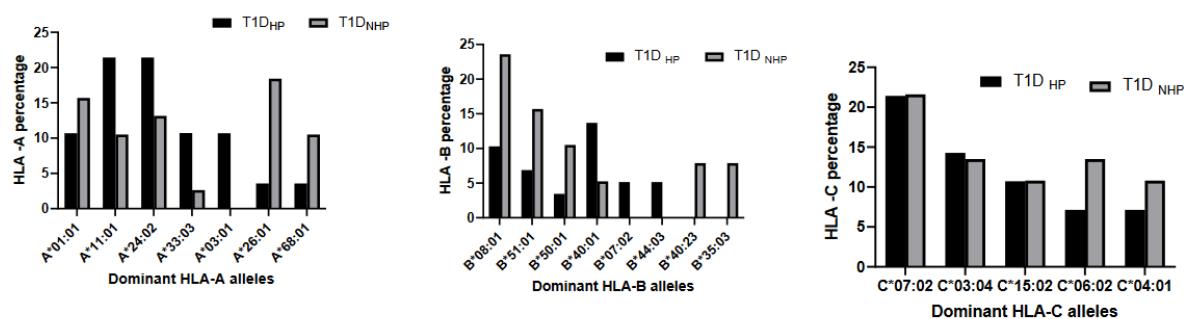
Conclusion: During the honeymoon phase, a reduction in proinflammatory cytokines alongside increased CD4+ and CD8+ EM, CM, TEMRA, Tregs, and B cell subsets suggest restoring of immune homeostasis, temporarily preserving beta-cell function through immune tolerance.



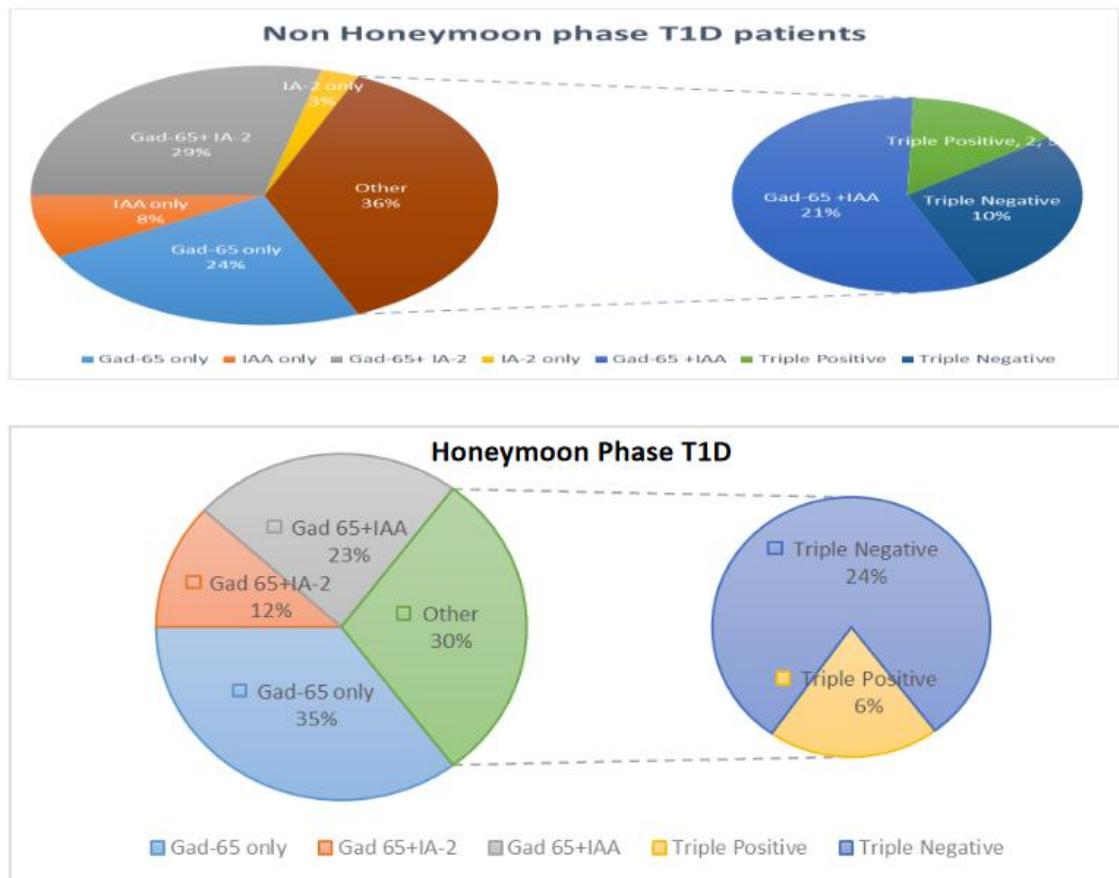


HLA phenotyping of 20 T1D_{HP} and 30 T1D_{NHP} patients

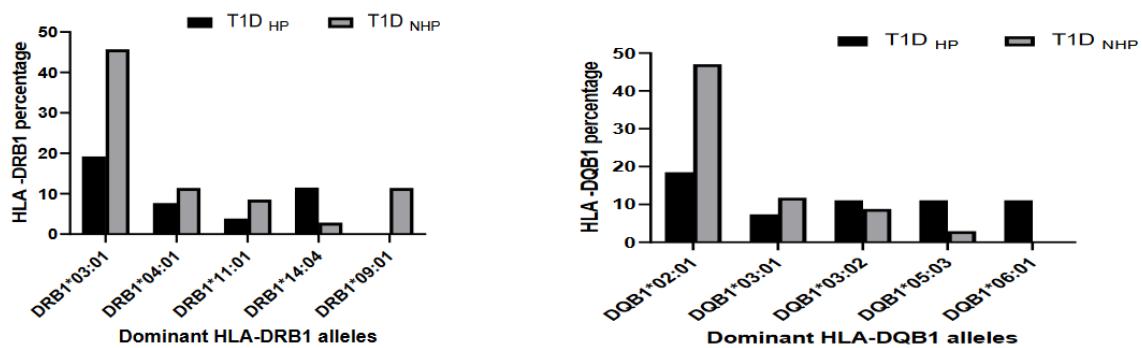
Frequency of Class-I HLA Phenotypes



Autoantibody Profile



Frequency of HLA Class-II phenotypes



BP 25: T-Cell Subset Dysregulation in Melioidosis: Comparative Analysis of Immune Responses in Patients and Healthy Controls

Supriyo Mukherjee, Sparsha Pallen, Sindhura Lakshmi Koulmane Laxminarayana, Piyush Behari Lal, Somashish Ghosh Dastidar, Chiranjay Mukhopadhyay, Ranita Ghosh Dastidar

KASTURBA MEDICAL COLLEGE, MANIPAL, MANIPAL ACADEMY OF HIGHER EDUCATION

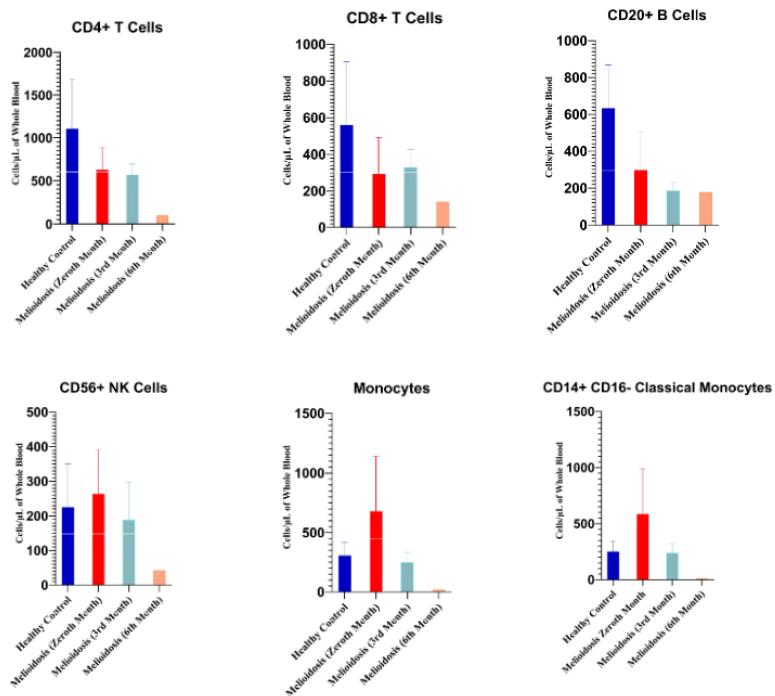
Background: *Burkholderia pseudomallei*, the causative agent of melioidosis, imposes a substantial health burden in regions like Southeast Asia and Northern Australia. While the role of T-cells in the immune response against this pathogen is recognized, the specific contributions of distinct T-cell subsets remain poorly elucidated. This study aimed to investigate and compare the distribution and activation of CD4+ and CD8+ T-cell populations, including regulatory T cells (Tregs) and distinct memory or effector phenotypes (Th1, Th2, Th17, Th22), as well as activation markers such as HLA-DR and CD38, in melioidosis patients versus age- and sex-matched healthy controls.

Methods: Peripheral blood samples from adult patients with melioidosis (n=19) and healthy controls (n=13) were analyzed for CD4+ and CD8+ T-cell subsets and their activation status within whole blood. Statistical analysis was performed using GraphPad Prism 10.2.0. Differences in T-cell subsets and activation marker expression between patient and control groups were evaluated using the Mann-Whitney U test for non-parametric data. A p-value of <0.05 was considered statistically significant.

Results: Preliminary data revealed significant alterations in the T-cell compartment among melioidosis patients. There was a marked reduction in the proportions of Th1(CXCR3+), Th17(CCR6+), and Th22(CCR10+) subsets, alongside decreased expression of activation markers HLA-DR and CD38 on CD4+ and CD8+ T-cells.

Conclusion: The dysregulation of T-cell subsets and reduced expression of crucial activation markers like HLA-DR and CD38 highlight the critical role of T-cell responses in combating *Burkholderia pseudomallei*. The reduced HLA-DR expression suggests impaired antigen presentation and a diminished ability to control inflammation. Similarly, the reduction in CD38 expression may indicate a compromised T-cell activation response, potentially contributing to the progression and severity of the disease. These findings provide important insights into the immunopathogenesis of melioidosis.

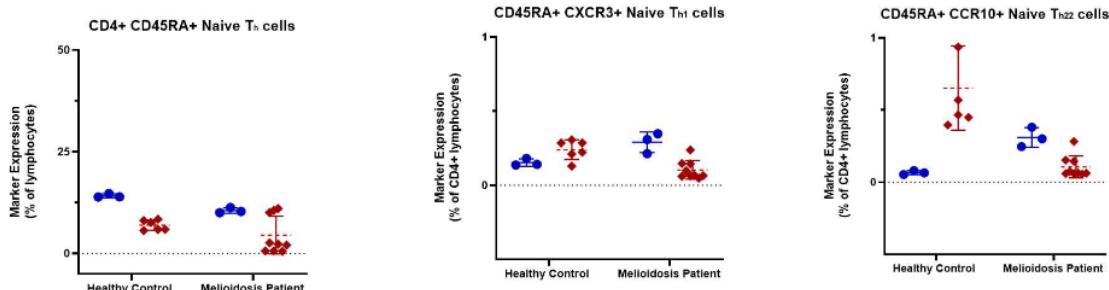
RESULTS



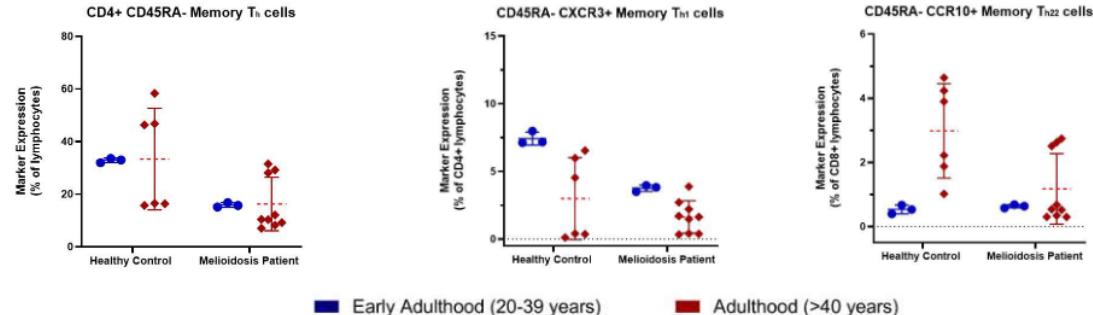
- CD4+ and CD8+ T Cells:** Higher in healthy controls, decline significantly in melioidosis patients, especially by the 6th month.
- CD20+ B Cells:** Gradual decrease in melioidosis patients over 6 months, higher in healthy controls.
- CD56+ NK Cells:** Slight decrease in melioidosis patients by the 6th month, similar across groups.
- Monocytes and Classical Monocytes:** Elevated in melioidosis patients, especially early on, decreases over time.

Results

Naïve CD4+ T cell subsets

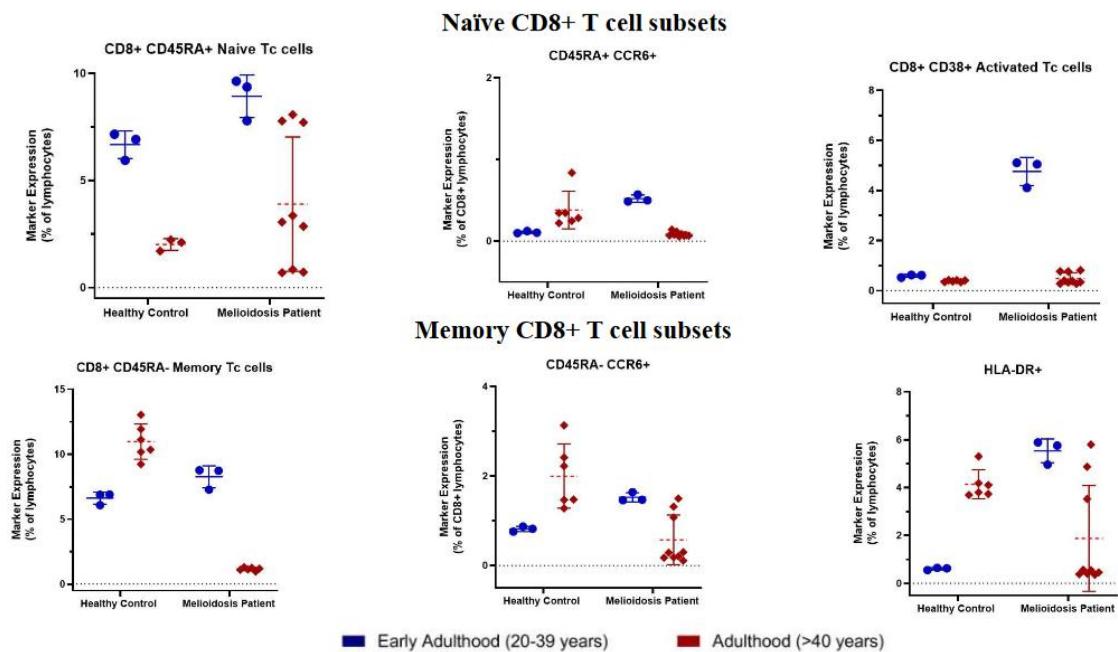


Memory CD4+ T cell subsets



■ Early Adulthood (20-39 years) ■ Adulthood (>40 years)

Results

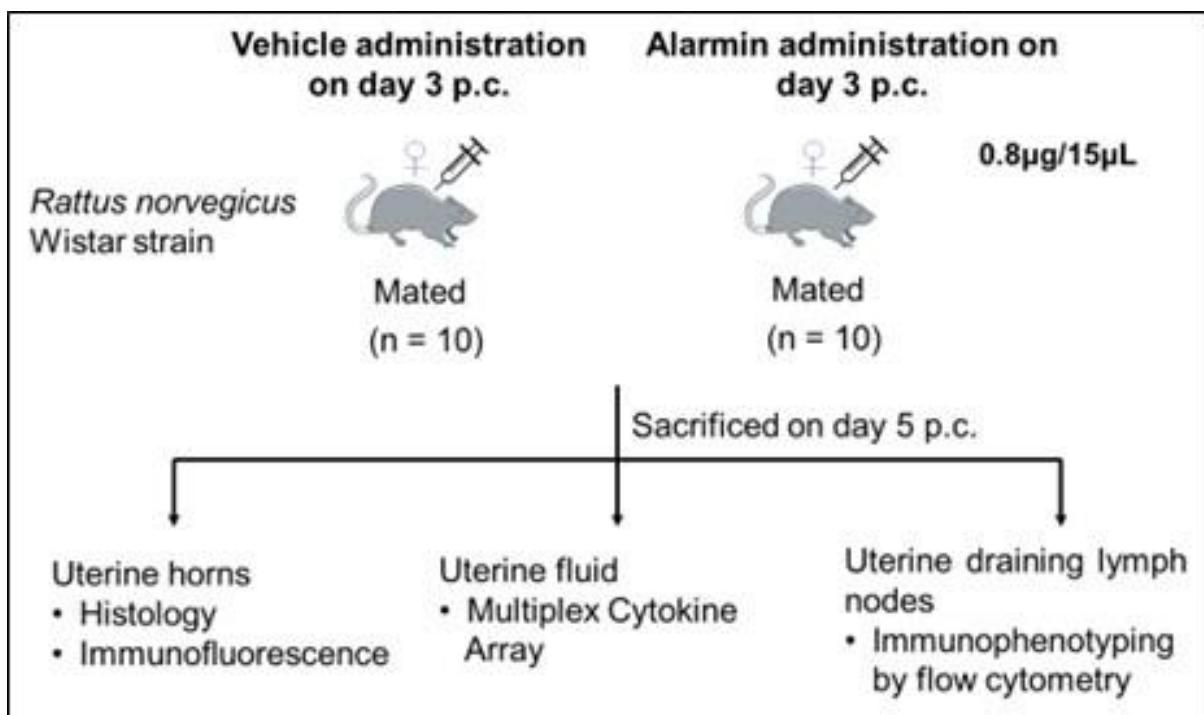


BP 26: Immune Environment in the Uterus of Rats Experiencing Implantation Failure Due to Excess of Alarmins

Rithika Rajendran, Sheetal Singhania, S.M. Metkari, Vainav Patel, Uddhav Chaudhari, Satish K. Adiga and Geetanjali Sachdeva

Indian Council of Medical Research-National Institute for Research in Reproductive & Child Health

Embryo implantation is an immunologically paradoxical event wherein a semi-allogeneic blastocyst invades into a receptive endometrium to access the maternal blood vessels, without being attacked by the maternal immune system. The breaching of the endometrium by the embryo is an inflammatory event. However, this inflammation needs to be regulated for a successful pregnancy. This study was undertaken to determine whether excess of extracellular high mobility group box 1 (HMGB1), a prototypical DAMP, in the uterine milieu alters the endometrial immune profile during implantation. Recombinant HMGB1 was administered exogenously in the uterine cavity of adult Wistar rats on day 3 post-coitum (p.c.). Control animals were administered with the same volume of saline. The animals were sacrificed on day 5p.c. Uterine fluid samples were collected and utilized for cytokine array. Part of the uterine horn was used for paraffin-block preparation for histology and immunofluorescence experiments. Single-cell suspension of endometrial cells was prepared to analyse the frequency of uterine immune cells by flow cytometry. Higher levels of HMGB1 in the uterine cavity led to reduced ($p=0.0004$) frequency of DBA-lectin+ uterine NK cells and increased ($p=0.0477$) frequency of uterine Tregs on day 5 p.c. While the total number of macrophages remained unchanged, the distribution of CD68+ cells ($p=0.0001$) and CD163+ M2 ($p=0.0036$) macrophages around the implantation sites was found to be altered in HMGB1-administered uterine horns. the frequency of M2 macrophages activated during tissue remodeling (CD68+C163+CD86+MHCIIlow) reduced ($p=0.0091$) in HMGB1-administered uteri. Multiplex cytokine array revealed increased IL-1 β ($p=0.0336$) and IL-5 ($p=0.0255$) levels post HMGB1-administration. Immunofluorescence of decidualization markers at the implantation sites revealed reduced localization of IGFBP1 ($p=0.0015$) and Prolactin ($p=0.0035$) in the HMGB1-administered uterine horns. Our investigations revealed that excess of HMGB1 in the uterine microenvironment during implantation result in impaired decidualization and implantation failure. This may be attributed to reduced frequency of uNK cells and impaired polarization of macrophages.



BP 27: The Impact of Bisphosphonates on the Osteoclast Cells of Osteogenesis Imperfecta Patients

Vandana Dhiman1, S K Bhadada*1, Nirmal G Raj2, Naresh Sachdeva1, D K Dhawan3

Post Graduate Institute of Medical Education and Research (PGIMER), Chandigarh, India

Background: Bisphosphonates (BPs) are widely used for the treatment of osteogenesis imperfecta (OI). However, prolonged use may be associated with suppression of bone turnover, the exact molecular mechanism of which is poorly understood. This study aimed to evaluate the effect of zoledronic acid (ZOL) on osteoclast precursors.

Methods: We recruited 10 OI patients and 5 healthy controls. Out of 10 OI, 5 had received <30 mg and 5 received 30 mg of ZOL. Peripheral blood mononuclear cells were isolated from the subjects. Caspase activity in monocytes (CD14+ and RANKL+) was measured in all groups. The bead-based protein multiplexing was done on the serum of OI patients by flow cytometry.

Results: Six of the OI patients had type IV, two had type III and one each had types I & II of OI, respectively. Radiographs showed “zebra stripe sign” and dense metaphyses, suggesting acquired osteosclerosis. Bone turnover markers (PINP and CTx) were suppressed in all OI patients compared to controls. Caspase-3 activity was significantly increased in osteoclast precursor cells at higher doses of BPs (>30 mg). The bead-based protein multiplexing reveal significantly higher expression of osteopontin (OPN) (OI: 37649.98 ± 53984 vs HC: 10883.54 ± 17561), RANKL (TRANCE) (OI: 692.55 ± 900.2 vs HC: 52.97 ± 15.89), and Tumor Necrosis Factor Alpha (TNF- α) (OI: 66.99 ± 94.24 vs HC: 8.31 ± 4.26).

Conclusion: Excessive use of ZOL in OI patients suppresses bone turnover markers (CTx, PINP) and also causes osteosclerosis. High dose ZOL inhibits osteoclastic activity (increased expression of caspase 3 activity) which in turn predisposes to atypical fractures and delayed fracture healing. Thus, this study will be helpful in developing future autologous immunotherapy-based treatment modalities for OI.

Figure: Radiograph of lower limb in 8 year old boy showed the presence of generalized osteosclerosis



Figure: Percentage of Caspase-3 % of osteoclast cells

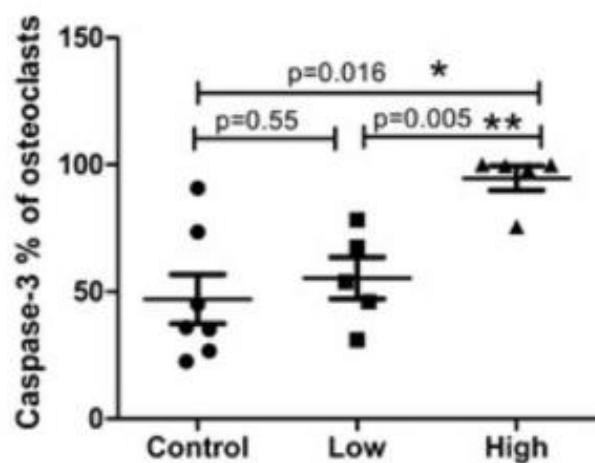


Figure: MFI (median) caspase-3 of osteoclast cells

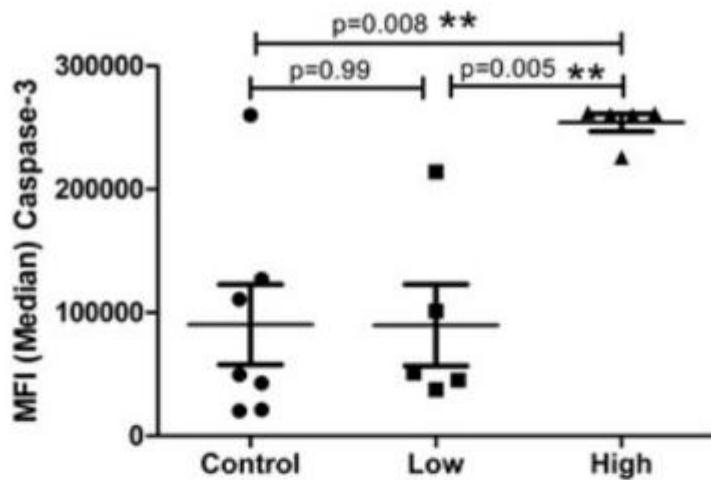
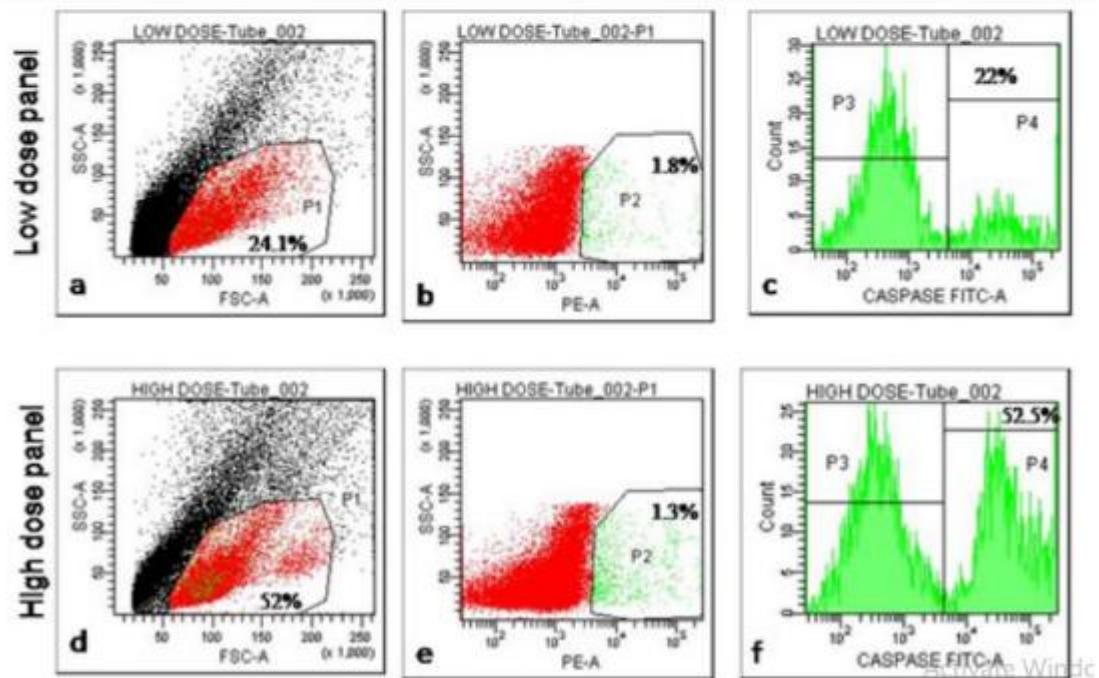


Figure: Representative flow cytometric plots showing the gating strategy for the analysis of RANK+Caspase+ cells



BP 28: Clinicopathological and immunophenotypic characteristics of T-prolymphocytic leukemia: experience from a tertiary care centre

Sananda Kumar, Nabhajit Mallik, Malvika Shastri, Parveen Bosse, Praveen Sharma, Sreejesh Sreedharanunni, Pankaj Malhotra, Man Updesh Singh Sachdeva

Postgraduate Institute of Medical Education and Research, Chandigarh

Background: T-prolymphocytic leukemia (T-PLL) is a rare and aggressive mature T-cell neoplasm of post-thymic origin. There is sparse clinico-pathological and immunophenotypic data of this entity from India.

Methodology: All cases diagnosed as T-PLL at our centre over the past 10 years were included, and their demographic characteristics, clinical features, hematological parameters and immunophenotypic findings were analysed.

Results: Thirteen cases of T-PLL were identified, out of which 7 (53.8%) were males and 6 (46.2%) were females. The median age of presentation was 58 years (range:40-85 years). Clinical details were available in 9/13 patients; four patients (44.4%) had hepatosplenomegaly, 2 (22.2%) had generalised lymphadenopathy, while one (11.1%) had skin involvement (infiltrative plaque on face). The mean TLC was $154.8 \times 10^9/L$ (range $11.0-433.6 \times 10^9/L$), mean hemoglobin was 110 g/L (range 75-141 g/L) and mean platelet count was $153.7 \times 10^9/L$ (range $12-384 \times 10^9/L$); leucocytosis, anemia and thrombocytopenia were present in 92.3%, 50% and 37.5% respectively. On flow cytometry, 40% cases showed downregulation of CD3, CD7 and CD2 were downregulated in only 1 case (7.6%) each, and all cases expressed CD5. 6 cases (46.1%) had a CD4 positive/CD8 negative profile, 6 cases (46.1%) were dual positive for CD4 & CD8, while 1 case (7.6%) was CD8 positive/CD4 negative. CD56 expression was seen in one case. Surprisingly, CD45 was almost completely negative in one case, and showed dim expression in a subset of leukemic cells in two cases (downregulation in 23% cases). Clonality was determined by TRBC1 assay and TCR gamma rearrangement assay in 4 cases each.

Conclusion: CD3 downregulation was the most common immunophenotypic aberrancy, and equal proportion of cases (46.1% each) were CD4 positive/CD8 negative and CD4/CD8 dual positive. A remarkable finding was CD45 downregulation in 23% cases, which may cause potential diagnostic confusion with T-ALL, underscoring the importance of careful clinical, morphological and immunophenotypic evaluation of this rare neoplasm.

BP 29: Prognostic and Diagnostic significance of MMP-2 and MMP-9 in NMIBC and MIBC patients

Rohit Siddhartha, Apul Goel, Atin Singhai, and Minal Garg

University of Lucknow

Background: Matrix metalloproteinases (MMPs) also known as matrixins are involved in the remodeling of extracellular matrix (ECM), regulation of cell expression, morphogenesis, wound healing, bone resorption, apoptosis, angiogenesis, and metastasis. MMP-2 and MMP-9 bring about major changes in the ECM, release of cell surface receptors, chemokines, aid tumor angiogenesis and metastasis in a multifactorial manner. Functional role of overexpressed MMP-2 and MMP-9 in the pathogenesis of two distinct subtypes of urothelial carcinoma of bladder (UCB), namely non-muscle invasive bladder cancer (NMIBC) and muscle invasive bladder cancer (MIBC) makes them promising candidate for investigating the diagnostic and prognostic significance of the disease.

Methods: Tumor tissue specimens/ sections were obtained from surgically resected NMIBC and MIBC patients. Transcriptomic analysis by RT-qPCR was performed to evaluate average fold change expression of MMP-2 and MMP-9 while their proteomic analysis was done by immunohistochemical staining, immunoblotting and zymography. The expression results were statistically analysed with patients' demographics using SPSS-20, Prism and validated by TCGA ,GEPIA2 databases.

Results: Transcriptomic and proteomic expressions of MMP-2 and MMP-9 were observed to be statistically relevant with tumor size, type, stage and tobacco chewing/ smoking history of NMIBC and MIBC patients. Log rank test along with Kaplan Meier analysis exhibited MMP-2 as the strong predictor of short overall survival, recurrence free survival in NMIBC cohorts while short overall and poor cancer specific survival in MIBC cohorts. Levels of MMP-9 were observed to be the significant predictor of poor recurrence free survival in NMIBC cohorts and poor progression free survival and short cancer specific survival in MIBC cohorts.

Conclusions: Elevated expressions of MMP2, MMP9 at message and proteomic level in the NMIBC, MIBC cohorts characterize them as potential biomarkers, however their analysis in bigger cohorts is required to validate their importance in the clinical management of bladder cancer patients.

BP 30: Understanding the Mesenchymal stem cells' role in uterine and intestinal tissue regeneration

Jayeeta Giri, Deepak Modi

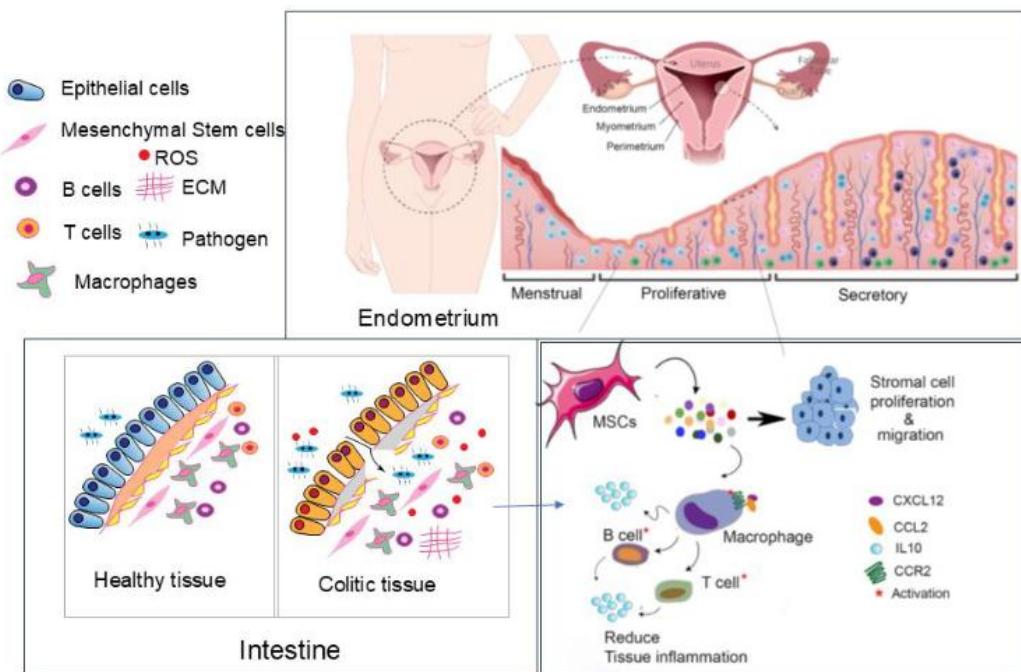
ICMR-National Institute for Research in Reproductive and Child Health

Introduction: Among the other tissue-resident adult stem cells, Mesenchymal stem cells (MSCs) are well characterized by their immune-modulatory and regenerative functionality. Impairment of tissue regeneration leads to fibrosis/ scar formation. To understand the mechanism of scar-free tissue repair by resident adult stem and immune cells, we selected intestine and endometrium tissues. These are the extremely regenerative organs in the human body. The intestine redevelops its lining, epithelium, every 3-4 days. The endometrium (the inner lining of the uterus) suffers monthly shedding and regeneration, pivotal for successful reproduction. I aim to identify the role of tissue-resident MSCs in the regulation of intestinal and endometrial homeostasis.

Methods & Results: We created colitis-mediated intestinal damage in mice and exogenously delivered MSCs to these colitis mice. We studied the immune phenotype of intestinal macrophage/ B or T cells. To characterize intestinal MSCs, we collected MSCs from mice intestines with a standard collagen digestion method and characterized them based on MSCs-specific markers. We measured R-spondin-3/Wnt5a expression, the two morphogens with known function in epithelial stem cell biology. We also measured Collagen 1 and 5 expression, components of fibrosis. To identify the role of MSCs in endometrium regeneration, MSCs, and stromal cells were collected from the menstrual blood of healthy women. Endometrial stromal cells were treated with MSCs-condition media. Stromal cell proliferation and random and collective migration were assayed using standard biochemical assays. MSC-CM-treated stromal cells' ability to reform the endometrial tissue was measured by tissue decellularization/recellularization technique.

Conclusion: MSCs derived secretome (MSC-CM) reduces tissue inflammation and induces tissue regeneration in the intestine and endometrium. Significance: MSC-CM can be a novel cell-free therapy against uterine/endometrium damage.

Graphical abstract:



Intestinal and endometrium Mesenchymal stem cells (MSCs) participate in tissue remodelling after any pathogenic insult or damage, to maintain tissue homeostasis.

BP 31: Immunophenotypic characterisation of leukemia using flow cytometry- a study of 30 cases

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Introduction: Leukemia is a diverse group of hematological malignancies, and accurate diagnosis is crucial for effective treatment. Immunophenotyping using flow cytometry is a powerful tool for distinguishing leukemia subtypes based on cell surface and intracellular antigen expression.

Aim: This study aimed to characterize the immunophenotypic profiles of 30 leukemia cases using flow cytometry to enhance diagnostic accuracy and inform therapeutic decisions.

Materials and Methods: The study included 30 cases of leukemia, encompassing acute and chronic forms from both myeloid and lymphoid lineages. Peripheral blood and bone marrow samples were analyzed using flow cytometry. Panels of monoclonal antibodies targeting specific antigens (e.g., CD34, CD13, CD19, CD33, CD3) were used to identify leukemic cells. The immunophenotypic results were correlated with clinical presentations and morphological findings.

Results: Distinct immunophenotypic patterns were observed across different leukemia subtypes. Acute myeloid leukemia cases showed predominant expression of myeloid markers such as CD13, CD33, and CD34, while acute lymphoblastic leukemia cases exhibited strong positivity for CD19 and CD10. Chronic leukemias displayed characteristic patterns aligning with their respective lineages. The data helped in refining diagnoses, particularly in cases with overlapping morphological features.

Conclusion: Immunophenotyping by flow cytometry is an invaluable tool for the precise characterization of leukemia, aiding in the differentiation of subtypes and guiding treatment decisions. This study reinforces the role of flow cytometry in improving diagnostic accuracy, particularly in cases where traditional methods are insufficient.

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