The Cytometry Society, India

www.cytometryindia.org
www.tcs.res.in

Flow Cytometry
From Basics to Multi-Omics

15TH Annual TCS Conference & Workshops

26th – 29th October 2023

JLN Auditorium
All India Institute of Medical Sciences

e - Souvenir

ADD MORE COLORS WITHOUT ADDING MORE CONCERNS

DxFLEX FLOW CYTOMETER

DxFLEX is a new clinical flow cytometry platform derived from the successful CytoFLEX. The advanced sensitivity and intuitive software DxFLEX makes flow cytometry routine for both novice and expert flow cytometry technicians and promotes standardization.

- Wavelength Division Multiplexing (WDM) detection modules offer superior signal resolution
- Up to 13 colors CE-IVD to get the most out of your specimen
- Improved resolution and linearity
- Dynamic compensation library for easy compensation setup
- Compact system to free up bench space
- Easy to learn software interface
- Simplified system settings, data acquisition & result export functions
- Different instrument configurations available with up to 3-laser and 13-color detection
- Optional DxFLEX Autoloader decreases the hands on time with multiple tube loading and plate loading*

Beckman Coulter India Pvt Ltd
3rd Floor, B-Wing, Art Guild House, Phoenix Market City, LBS Road, Kurla (West), Mumbai - 400 070
Ph: +91 90828 43198 | +91 91368 49723 | Email: marketing.india@beckman.com | Website: www.beckman.com

For Beckman Coulter's worldwide office locations and phone numbers, please visit Contact Us at beckman.com

*Plate adapter: Regulatory status differs by region, please consult your local sales representative.

The Cytometry Society-India

www.tcs.res.in

Organizing Committee

WELCOMES YOU TO

15th Annual Conference & Workshops

26th -29th October, 2023

Theme: From Basics to Multi-Omics

Venue

All India Institute of Medical Sciences, New Delhi

eSOUVENIR

Table of Contents

The Cytometry Society-India
15th Annual Conference & Workshops
26th -29th October, 2023
Theme: From Basics to Multi-Omics

Executive Committee
2021-23 (Current)

President
Prof Ritu Gupta
All India Institute of Medical Sciences, New Delhi

Vice-President (Clinical)
Dr. Anil Handoo
BLK Super Speciality Hospital New Delhi

Vice-President (Research)
Prof Urmi Chatterji
University of Calcutta

Joint Secretary
Dr. Prashanth Tembhare
ACTREC, TATA Memorial Centre, Navi Mumbai

General Secretary (Clinical)
Dr. Kunal Sehgal
Sehgal Path Lab Pvt Ltd, Mumbai

General Secretary (Research)
Dr. Vainav Patel
ICMR-NIRRH, Mumbai

Treasurer
Dr. Khaliqur Rahman
SGPGIMS, Lucknow

Member
Dr. Nitin Dayal
Max Super Speciality Hospital, Delhi

Member
Ms. Maya Ravishankar Gupta
ICMR-National Institute of Immunohematology,Mumbai

Member
Dr. Sachin Jain
Fortis Memorial Research Institute, Gurgaon

Member
Dr Sanjeev Kumar Gupta
All India Institute of Medical Sciences, New Delhi

Member
Dr. Naresh Sachdeva
Post Graduate Institute of Medical Education and Research, Chandigarh

Member
Dr.Karthik Bommannan
B.K.Cancer Institute (W.I.A.), Chennai

Member
Dr. Ananthvikas Jayaram
Neuberg Anand Reference Laboratory, Bangalore

Office Bearers

M Illias Shareef
CCMB, Hyderabad

G Srinivas
CCMB, Hyderabad

The Cytometry Society-India
15th Annual Conference & Workshops
26th -29th October, 2023
Theme: From Basics to Multi-Omics

Executive Committee
2023-25

President
Prof Urmi Chatterji
University of Calcutta

Vice-President (Clinical)
Dr. Anil Handoo
BLK Super Speciality Hospital
New Delhi

Vice-President (Research)
Dr. Vainav Patel
ICMR-NIRRH,
Mumbai

Joint Secretary
Dr. Khaliqur Rahman
SGPGIMS, Lucknow

General Secretary (Clinical)
Dr. Kunal Sehgal
Sehgal Path Lab Pvt Ltd,
Mumbai

Treasurer
Dr. Prashanth Tembhare
ACTREC, TATA Memorial
Centre, Navi Mumbai

Member
Ms. Maya Ravishankar Gupta
ICMR-National Institute of
Immunohematology,Mumbai

Member
Dr. Nitin Dayal
Max Super Speciality Hospital,
Delhi

Member
Dr Sanjeev Kumar Gupta
All India Institute of Medical Sciences, New Delhi

Member
Dr. Sachin Jain
Fortis Memorial Research Institute, Gurgaon

Member
Dr.Karthik Bommannan
B.K.Cancer Institute (W.I.A.), Chennai

Member
Dr. Naresh Sachdeva
Post Graduate Institute of Medical Education and Research, Chandigarh

Member
Dr. Ananthvikas Jayaram
Neuberg Anand Reference Laboratory, Bangalore

Member
Dr.Vikrant Bhor
ICMR-NIRRCH
Mumbai

Office Bearers

M Illias Shareef
CCMB, Hyderabad

G Srinivas
CCMB, Hyderabad

The Cytometry Society-India
15th Annual Conference & Workshops
26th -29th October, 2023
Theme: From Basics to Multi-Omics

Organizing Committee

President
Prof Ritu Gupta
All India Institute of Medical Sciences, New Delhi

Vice-President (Clinical)
Dr. Anil Handoo
BLK Super Speciality Hospital New Delhi

Member
Dr. Nitin Dayal
Max Super Speciality Hospital, Delhi

Member
Dr. Sachin Jain
Fortis Memorial Research Institute, Gurgaon

Member
Dr Sanjeev Kumar Gupta
All India Institute of Medical Sciences, New Delhi

Dr. Smeeta G.
All India Institute of Medical Sciences, New Delhi

Dr. Gurvinder Kaur
All India Institute of Medical Sciences, New Delhi

Dr. Deepshi Thakral
All India Institute of Medical Sciences, New Delhi

Dr. Lata Rani
All India Institute of Medical Sciences, New Delhi

Dr. Rupesh Srivastava
All India Institute of Medical Sciences, New Delhi

Dr Sandeep Rai
All India Institute of Medical Sciences, New Delhi

Dr. Kamal Gulati
All India Institute of Medical Sciences, New Delhi

Mr. G.Srinivas
CCMB, Hyderabad

Dr. Rahul Sharma
All India Institute of Medical Sciences, New Delhi

Mrs. Saroj Singh
All India Institute of Medical Sciences, New Delhi

DR. SUSHMA BHATNAGAR
डॉ. सुषमाभटनागर
CHIEF, DR. B.R.A.I.R.C.H.
प्रमुख, डॉ. भी.रा.अं., सं.रो.कै.अ.
PROF. & HEAD, ONCO-ANAESTHESIA & PALLIATIVE MEDICINE
आचार्यएवंअध्यक्षअर्बुद-संवेदनाहरणएवंप्रशामकचिकित्साविभाग
AIIMS, NEW DELHI-110029
अ.भा.आ.स., नईदिल्ली-110029

MESSAGE
CHIEF, DR. B.R.A IRCH, AIIMS

It gives me great pleasure to learn that the 15th Annual Meeting of The Cytometry Society (TCS) of India is being hosted by the Laboratory Oncology at Dr BR Ambedkar Institute Rotary Cancer Hospital, All India Institute of Medical Sciences.

Flow Cytometry is one of the landmark discoveries in science as it integrates both basic and clinical applications. It has an upfront applied value in diagnostics and therapeutics. This conference based on 'Basic to Multi-Omics' will provide a platform for sharing knowledge and expert opinion in recent innovations and advancements in this field both for basic and clinical researchers.

I would like to congratulate the organizing team for organizing such an important event and express my best wishes for a great success.

Sushma Bhatnagar

Dr. Sushma Bhatnagar

Prof. Ritu Gupta
Laboratory Oncology
Dr. B.R.A. Institute Rotary Cancer Hospital
All India Institute of Medical Sciences
Ansari Nagar, New Delhi – 110029

MESSAGE FROM THE DESK OF PRESIDENT, THE CYTOMETRY SOCIETY (INDIA)

It is an honor for me and my organizing team to organize and invite you all to the 15th Annual Meeting of The Cytometry Society (TCS) of India is being hosted by the Laboratory Oncology at Dr B.R. Ambedkar Institute Rotary Cancer Hospital, All India Institute of Medical Sciences.

Recent years have witnessed path breaking discoveries in multi-omics as well as flow cytometry. The journey from basic flow cytometry to multi color multi dimensional to next generation flow and its amalgamation with AI has been a challenging task and has unfolded several new mysteries to be resolved. While the technology has geared us with unbelievable sensitivities, we are still learning to utilize its strength to the fullest in basic and clinical research as well as for better patient care. We are now equipped to provide better diagnostics and therapeutics using newer developments and analysis protocols that have evolved and been custom designed for various applications of flow cytometry.

The theme of this conference is 'From Basics to Multi-Omics' where many international expert stalwarts in flow cytometry will deliberate and share their knowledge on different aspects of flow cytometry. The scientific program is highly motivating and the participants will benefit immensely from the conference and can earn CME points from the Delhi Medical Council.

Of note, there will also be parallel training workshops on Basics of Flow, Cell Sorting, MRD, Multi-Omics, Primary Immunodeficiencies and Transplant Immunology prior to the conference where participants will get a chance to interact closely with the faculty and get geared to learn more from the experts during the conference.

I take this opportunity to acknowledge all the support received from AIIMS and TCS for their hard work and efforts in organizing this conference.

Looking forward to welcome you all to this scientific feast and your stay in Delhi.

Ritu Gupta

Prof Ritu Gupta
President, The Cytometry Society (India)

MESSAGE FROM THE DESK OF ORGANIZING SECERETARIES

Dear Friends,

It gives us utmost pleasure to welcome you all to 15th Annual TCS conference & Workshops 2023, being held in Delhi, the capital city of India, often referred to as the "Heart of India". This beautiful historic city has a legacy of multi-cultural heritage amalgamating to modernity.

Since the time we were entrusted with the task of organising this conference we have been thrilled and excited. We, along with the TCS executive committee and organizing team, have put in sincere efforts to have an academic extravaganza covering both basic and clinical aspects of cytometry science, as well as, ensure a peek into the future of flow cytometry covering its utility in "omics". We truly believe this meeting shall provide everyone a great opportunity to share views, studies, and research observations in the field of flow cytometry with all our fellow colleagues and to listen to best of the national & international speakers carefully selected by the most experienced team of scientific committee.

Along with the enlightening scientific content the delegates would have several other interesting sites to visit, in and around Delhi. Visit to sites of historical importance, savouring the Mughlai and other culinary delights of old Delhi, are just a few of the many.

We look forward to an enriching experience for all and wish you a successful, enjoyable & memorable conference. We hope that you will return with sweet memories of this conference and would wish to visit this place again.

Looking forward to see you all soon.

Dr. Nitin Dayal
Organizing Secretary
15th Annual TCS Conference & Workshops

Dr. Anil Handoo
Organizing Secretary
15th Annual TCS Conference & Workshops

Scientific Program: Day 1		
28th October, 2023		
Plenary Session		
Session Chairs - Prof. Tejinder Singh & Prof. DK Mitra		
Time Slot	**Topic/Event**	**Speaker**
9:00 - 9.45	Journey of Flow Cytometry in India	Dr Sumeet Gujral
9:45 - 10:15	**Inauguration & Welcome Address**	
10:15-10:45	**TEA/COFFEE BREAK**	
Session 1: Chaired By - Prof. Sameer Bakhshi & Prof. Punit Kaur		
10:45 -11:30	The interplay between genetics and lineage in acute leukaemia diagnosis	Dr Sindhu Cherian
11:30 -12:00	Dissecting hematological malignancies: Flow-cytometry & Single-cell sequencing	Dr. Ritu Gupta
12.00-12.30	The Ooh, The Aah and The Ouch of - Assay validation of quantitative rare event data acquisition	Dr Urmi Chatterjee
12.30 –13:00	Cytometry Analysis and Deep Learning	Dr. Anubha Gupta
13.00 –3:30	Ethical issues/Professional Misconduct	Dr Girish Tyagi/ Dr Arun Gupta
13.00 –14:00	**LUNCH BREAK**	
Session 2: Chaired By - Prof. Vibha Tandon & Prof Kalpana Luthra		
14:00-14:30	The cellular path to pathogenesis- A tale of 3 viruses	Dr Vainav Patel
14:30-15:00	Flow-cytometry approach for diagnosis, classification and monitoring of T-cell CLPD	Dr Prashant Tembhare
15:00-15:30	Chasing the immune checkpoint molecules in HIV-TB co-infection	Dr Sunil Arora
15:30-16:00	The Future of Discovery: Bridging the intersection of spatial imaging with high parameter spectral flow cytometry	Dr. Robert Balderas
16:00-16:15	**TEA/COFFEE BREAK**	
Session 3: TCS Best Paper Awards' Presentations Chaired By - Prof Jyoti Kotwal, Prof. Amar Das Gupta, Prof. H. Krishnamurthy		
16:15-16:30	TCS Best Paper Awards' Presentation-Basic Science	
16:30-16:45	TCS Best Paper Awards' Presentation- Clinical	
Session 4: Poster Evaluation & TCS EC Meeting Poster-Clinical: Judge- Dr. Arul Selvi, Dr. Manupdesh Sachdeva, Dr. Nitin Dayal Poster-Basic: Judge- Dr. Gurvinder Kaur, Dr, Mayank Singh, Maya R. Gupta		
16.45– 17:30	Poster Session Evaluation & Networking	
17:30 –18:15	TCS Executive Committee Meeting	For EC members only

Scientific Program: Day 2		
29th October, 2023		
Plenary Session		
Chaired By - Prof. Sanjeev K Gupta, Dr. Tanima Dwivedi, Dr. Amrita Saraf		
Time Slot	**Topic/Event**	**Speaker**
9:00 -9.45	Getting Ready for MRD Assessment in the Era of Immunotherapy	Dr. Sindhu Cherian
9.45-10.30	Special Session Chaired By - Dr Anil Handoo & Dr Sindhura Laxmi	Dr H Krishnamurthy
10:30-11:00	**TEA/COFFEE BREAK**	
Session 1: Parallel Sessions - Basic Sciences (Conference Hall, First Floor) **Chaired By - Dr. Vinita Agarwal & Dr. Deepshi Thakral**		
11:00-11:20	Tumour associated MDSCs in WM	Dr Vaishali Bhardwaj
11:20-11:40	Disease Biology at Single Cell Resolution	Dr. Sunil Raghav
11.40 -12.00	Unlocking role of proinsulin in type-1 diabetes; from a key autoantigen to a primary therapeutic target	Dr. Naresh Sachdeva
12.00-12.20	Mesenchymal Stem Cells-Platelet interactions & wound healing	Dr. Anugya Bhatt
Session 1: Parallel Sessions - Clinical Sciences (JLN Auditorium) **Chaired By - Dr. Manu Goyal, Dr. Namrata Awasthi, Dr. Chandan Kumar**		
11:00-11:20	Translational approach for assay menu expansion in a clinical flow cytometry lab	Dr. Paresh Jain
11:20-11:40	Immunophenotype variants of normal populations- Circumventing pitfalls in MRD reporting	Dr. Kunal Sehgal
11.40 -12.00	Diagnosis beyond typical immunophenotypic fingerprint entities - Myelodysplasia - A Pattern Approach	Dr. P.G.Subramanian
12.00-12.20	Going beyond conventional flow cytometric testing- Platelet Activation Tests	Dr. Rutvi Dave
12.20–13:00	**General Body Meeting of TCS**	
13.00–14:00	**LUNCH BREAK**	
Session 2: Parallel Sessions (Oral Papers) **Clinical Science- Judges: Dr. Mrinalini Kotru, Dr. Tushar Sehgal, Dr. Sarika Singh** **Basic Sciences- Judges: Prof. Sunil Raghav, Dr. Surender Sherawat, Dr. Priyavadhana**		
14:00-15:30	Oral Papers-Clinical Sciences (JLN Auditorium)	
14:00-15:30	Oral papers– Basic Sciences (Conference Hall)	
15:30-15:45	**TEA/COFFEE BREAK**	
Session 3: Chaired By—Dr. Garima Jain		
15:45-16:00	Single Cell Sorting	Dr. Sushilkumar D. Ramdasi
16:00-16:20	PCPRO and Myeloma-MRD screening	Dr. Dragan Jevremovic
16:20-16:30	Flow cytometry approach for rare event analysis	Dr. Pradeep Rai
16.30 -17:00	**AWARDS, VALEDICTORY & TEA**	

15th TCS Annual Conference & Workshops 2023
Theme: Workshop on Measurable Residual Disease (MRD)
Venue: Dr. Ramalingaswamy Board Room, AIIMS, New Delhi
Dates: 26th & 27th October, 2023

Workshop Coordinators: Dr. Sachin Jain & Dr. G. Smeeta

FACULTY

Dr. Sindhu Cherian [LEAD FACULTY]
Associate Director of the Hematopathology Laboratory at the University of Washington
Dr Ritu Gupta
Professor, Laboratory Oncology, AIIMS, New Delhi

Scientific Program: Day 1

26th October, 2023

Session 1: 9:00 - 11:30

Time	Topic	*Speaker*
09:00 - 09:15	Importance of MRD in Hematological Malignancies	Dr. Ranjit K Sahoo
09:15 - 10:45	Introduction to MRD: Basics & Methods of MRD monitoring	Dr. Sindhu Cherian
10:45 - 11:30	Assay Performance Characteristics in Flow cytometry	Dr Anil Handoo
11:30 - 11:45	**TEA/COFFEE BREAK**	
Session 2: 11:45 - 13:30		
11:45 - 12:15	Approach to B ALL MRD: Panel design, template generation, internal controls & analysis	Dr. K. Bommannan
12:15 - 13:30	B-ALL MRD - Concepts & Case Studies	Dr G. Smeeta/Dr Nupur Das
13:30- 14:00	**LUNCH BREAK**	
Session 3: 14:00 - 15:30		
14:00 - 14:30	Approach to T ALL MRD: Panel design, template generation, internal controls & analysis	Dr Jasmita Das
14:30 - 15:30	T-ALL MRD: Concepts & Case Studies	Dr. M. Sachdeva
15:30 - 15:45	**TEA/COFFEE BREAK**	
Session 4: 15:45 - 17:15		
15:45 - 16:30	Approach to Myeloma MRD: Panel design, template generation, internal controls & analysis	Dr. Ritu Gupta
16:30 - 17:15	Myeloma MRD - Concepts & Case Studies	Dr. Khaliq-Ur-Rahman

Workshop Coordinators: Dr. Sachin Jain & Dr. G. Smeeta

FACULTY

Dr. Sindhu Cherian [LEAD FACULTY]
Associate Director of the Hematopathology Laboratory at the University of Washington
Dr Ritu Gupta
Professor, Laboratory Oncology, AIIMS, New Delhi

Scientific Program: Day 2

27th October, 2023

Session 1: 9:00 - 11:15

Time	Topic	*Speaker*
09:30 - 09:45	Re-cap of Day 1	Dr. Anil Handoo
09:45 - 10:15	Approach to CLL/Hairy Cell Leukemia MRD: Panel design, template generation, internal controls & analysis	Dr. Prashant Tembhare
10:15 - 11:15	CLL/Hairy Cell Leukemia MRD - Concepts & Case Studies	Dr. Naveen Aggarwal Dr. Prashant Tembhare
11:15 - 11:30	**TEA/COFFEE BREAK**	
Session 2: 11:30 - 13:30		
11:30 - 12:00	Approach to B ALL MRD: Panel design, template generation, internal controls & analysis	Dr. Sindhu Cherian
12:00 - 13:00	AML MRD - Concepts & Case Studies	Dr. Tina Dadu
13:00-13:30	Challenges in MPAL Through Case Studies	Dr. Sanjeev K. Gupta
13:30- 14:00	**LUNCH BREAK**	
Session 3: 14:00 - 16:00		
14:00 - 15:00	Infinicyt Software	Dr. Sachin Jain
15:00 - 15:30	Report formats - What's it that makes a good report?	Dr. Kunal Sehgal
15:30 – 16:00	Conclusion, Feedback and plans for subsequent meetings	All Faculty
15:30 - 15:45	**TEA**	

15th AIIMS-TCS Annual Conference & Workshops 2023
Theme: Advancing Research Through Cell Sorting
Venue: Central Core Research Facility (CCRF)
& SET Studio 1, AIIMS, New Delhi
Date: 26th October 2023

Workshop Coordinators: Dr. Rupesh Srivastava; Dr. Rahul Sharma; Dr. Sandeep Rai; Mr. G. Srinivas

FACULTY

Dr. Kalpana Luthra
Biochemistry, AIIMS, New Delhi
Dr. Rupesh Shrivastava
Department of Biotechnology, AIIMS, New Delhi
Dr. Rahul Sharma
Centralized Core Research Facility (CCRF), AIIMS, New Delhi
Dr. Sandeep Rai
Technical Officer, Laboratory Oncology, Dr. BRA IRCH, AIIMS, New Delhi
Mr. G Srinivas
Senior Technical Officer, Center for Cellular & Molecular Biology (CCMB), Hyderabad

Scientific Program

Registration: 8:45 - 9:15

	Session 1: 9:15 - 11:30 (SET Studio 1)	
Time	**Topic**	***Speaker***
09:15 - 09:30	Welcome Address	Dr. D.K. Mitra
09:30- 10:00	Introductory talk: Unlocking the Power of Cell Sorting	Dr. Kalpana Luthra
10:00 - 11:00	Cell Sorting Strategies: Practical & Technical perspective	Dr. Vinay Gupta
11:00 - 11:30	Question & Answer	All Faculty
11:30 - 11:45	**TEA/COFFEE BREAK**	
	Session 2: 11:45 - 13:30	
11:45 - 13:30	Sample Processing (Human Blood sample)	All Faculty
13:30 - 14:00	**LUNCH BREAK**	
	Session 3: 14:00 - 16:00	
14:00 - 15:30	Demonstration of cell sorting (4 way /single cell) & purity check	All Faculty
15:30 - 15:45	Troubleshooting, Do's & Don'ts in cell sorting	All Faculty
15:45 - 16:00	**Conclusion & Feedback**	Organizing Team
16:00 - 16:30	**TEA**	

15th TCS Annual Conference & Workshops 2023
Theme: Transplant Immunology
Venue: CMET, AIIMS, New Delhi
Date: 26th October 2023

Workshop Coordinators: Dr. Gurvinder Kaur & Dr. Nitin Dayal

FACULTY

Dr. Dolly Daniel
Head, Department of Immunohaematology & Transfusion Medicine, CMC Vellore
Dr. Aseem Tiwari
Director, Department of Transfusion Medicine, Medanta -The Medicity, Gurugram
Dr. Nitin Dayal
HOD Hematology, Max Super Speciality Hospital, Saket, New Delhi
Dr. Gurvinder Kaur
Scientist, Laboratory Oncology, AIIMS New Delhi
Dr. Mohit Chowdhary
Head, Department of Transfusion Medicine, Indraprastha Apollo Hospital, New Delhi

Scientific Program

Registration: 8:45 - 9:00

Session 1: 9:00 - 11:30

Time	Topic	Speaker
09:00 - 10:00	Evolution of Transplant Immunology	Dr. N.K. Mehra
10:30- 10:30	An approach to Antibody testing in transplantation	Dr. Dolly Daniel
10:30 - 11:00	CDC Crossmatch - Principle, methodology, analysis, interpretation, modifications & pitfalls	Dr. Aseem Tiwari
11:00 - 11:30	CDC Crossmatch - Concepts & Case Studies	Dr. Gurvinder Kaur
11:30 - 11:45	**TEA/COFFEE BREAK**	
Session 2: 11:45 - 13:30 **Session Chair: Dr. Nidhi Mehta**		
11:45 - 12:15	Flow Cytometry Crossmatch - principle, methodology, analysis, interpretation, modifications & pitfalls	Dr. Nitin Dayal
12:15 - 12:45	Flow Crossmatch - Case Studies	Dr. Gurvinder Kaur
12:45 - 13:30	The Good, The Bad and The Ugly of Luminex Crossmatch - principle, methodology, analysis, interpretation, and Cases	Dr. Mohit Chowdhary
13:30 - 14:00	**LUNCH BREAK**	
Session 3: 14:00 - 16:00		
14:00 - 14:30	HLA Antibody screening PRA - Flow cytometry Vs Luminex	Dr. Aseem Tiwari
14:30 - 15:30	SAB - principle, methodology, analysis, interpretation	Dr. Dolly Daniel
15:30 - 15:45	**TEA**	
15:45 - 17:00	Case Studies	All faculty

15th TCS Annual Conference & Workshops 2023
Theme: Basics of Flow Cytometry
Venue: FACS Academy Jamia Hamdard, New Delhi
Dates: 26th October, 2023

Workshop Coordinators: Dr. Vivek Singh & Mr. Swapnil Walke

FACULTY

Dr H Krishnamurthy (**Lead Faculty**)
Scientific Officer 'G', National Institute of Biological Sciences (NCBS), Tata Institute of Fundamental Research, Bengaluru
Dr Hemant Agrawal
Director, Flow Cytometry Solutions Pvt. Ltd.
Dr. Vivek Singh
Scientist, Laboratory Oncology, Dr. BRA-IRCH, New Delhi
Mr. Swapnil Walke
Senior Application Manager, BD Biosciences

Scientific Program

Registration: 8:45 - 9:30		
	Session 1: 9:30 - 11:30	
Time	**Topic**	**Speaker**
09:30 - 09:45	Welcome & Introduction	Dr. Vivek Singh
09:45- 11:30	Basic concepts of Flow Cytometry	Dr. H. Krishnamurthy
11:30 - 11:45	**TEA/COFFEE BREAK**	
	Session 2: 11:45 - 13:30	
11:45 - 12:45	Concept of Instrument QC	Dr. Swapnil Walke
12:45 - 13:30	Flow Cytometry Data Analysis and Presentation	Dr. Hemant Agrawal
13:30 - 14:00	**LUNCH BREAK**	
	Session 3: 14:00 - 16:00	
14:00 - 15:00	Instrument overview & QC	Dr. Manisha Suthar
15:00 - 15:30	Voltage optimization and compensation	Dr. H. Krishnamurthy Mr. Swapnil walke
15:30 - 16:30	Six-Color T-B-NK data acquisition and analysis	Dr. Manisha Suthar Mr. Swapnil Walke
	TEA	

15th AIIMS-TCS Annual Conference & Workshops 2023
Theme: Multi-Omics at Single Cell Level
Venue: Central Core Research Facility (CCRF)
& Set Studio 1, AIIMS, New Delhi
Date: 27th October 2023

Workshop Coordinators: Dr. Deepshi Thakral; Dr. Lata Rani; Dr. Murali Manohar*

FACULTY

Dr. Ritu Gupta*
Laboratory Oncology, Dr. BRA-IRCH, AIIMS, New Delhi
Dr. Arindam Maitra
Associate Director, NIBMG, Kalyani, West Bengal
Dr. Rajesh Pandey
Principal Scientist, CSIR-IGIB, New Delhi
Dr. Deepshi Thakral
Senior Scientist, Laboratory Oncology, Dr. BRA-IRCH, AIIMS, New Delhi
Dr. Lata Rani
Scientist, Centralized Core Research Facility (Genomics), AIIMS, New Delhi
Dr. Murali Manohar*
Scientist, Laboratory Oncology, Dr. BRA-IRCH, AIIMS, New Delhi

Scientific Program

Registration: 8:45 - 9:15 (SET Studio 1)

Session 1: SET Studio 1; Chaired By: Prof. Madhulika Kabra

Time	Topic	Speaker
09:15 - 09:30	Welcome Address	Dr. Ritu Gupta
09:30- 10:00	Single Cell Omics facilitating Canonical and Non-Canonical understanding of the host immune response	Dr. Rajesh Pandey
10:00 - 10:45	Our journey from bulk to single-cell transcriptomics in oral cancer: Novel insights and new horizons	Dr. Arindam Maitra
10:45 - 11:30	**TEA/COFFEE BREAK**	
	Session 2: Parallel Sessions: BD Rhapsody Workflow (SET Studio 1)	
11:30 - 11:45	Overview of the workshop	Dr. Deepshi Thakral
11:45 - 12:30	Latest trends in Single Cell Multi-Omics and its applications	Dr. Romsha Kumar
12:30 - 13:00	Tips and tricks of sample preparation	All Faculty
	Session 2: Parallel Sessions: 10X Sequencing workflow (CCRF)	
11:30 - 11:45	Overview of the workshop	Dr. Lata Rani
11:45 - 12:30	Biology at True Resolution: Resolve biological complexity with Single Cell Solutions	Dr. Sachin Minhas
12:30 - 13:00	Tips & Tricks of Sample Preparation	Santosh Sarma
13:00 - 14:00	**LUNCH BREAK**	
14:00 - 16:00	**Session 3: Parallel Sessions (Wet Lab Demonstration)**	All Faculty
16:00 - 16:15	**TEA/COFFEE BREAK**	
	Session 4: Parallel Sessions: BD Rhapsody Data Analysis (SET Studio 1)	
16:15 - 17:00	Data Visualization and analysis	Dr. Xiaohui Man
	Session 4: Parallel Sessions: 10X Data Analysis (CCRF)	
16:15 - 17:00	Data Visualization and analysis	Anuja Mishra
17:00 - 17:15	**Conclusion & Feedback**	All faculty

* Supported by Research Grant from SERB, DST (SPG/2021/004460)

15th TCS Annual Conference & Workshops 2023
Theme: Primary Immunodeficiency Diseases
Venue: CMET, AIIMS, New Delhi
Date: 27th October 2023

Workshop Coordinators: Dr. Sabina Langer & Dr. Ananthvikas Jayaram

FACULTY

Dr. Manisha Madkaikar
Scientist G & Director, ICMR National Institute of Immunohaematology, MUMBAI
Dr. Nita Radhakrishnan
Head of Pediatric Hematology Oncology, Post Graduate Institute of Child Health, Noida, UP
Dr. Sabina Langer
Sr. Consultant Hematology, Sir Ganga Ram Hospital, New Delhi
Dr. Ananthvikas Jayaram
Pathologist. Neuberg Anand Reference Laboratory, Bangaluru
Dr. Manas Kalra
Sr. Consultant, Paediatric Haemto-Oncology, Sir Ganga Ram Hospital, New Delhi

Scientific Program

Registration: 8:45 - 9:15

Session 1: 9:15 - 11:30

Time	Topic	Speaker
09:15 - 09:30	Welcome Address	Dr. Sabina Langer
09:30- 10:00	Introduction to IEIs with emphasis on lymphocyte disorders	Dr. Nita Radhakrishnan
10:00 - 10:45	The role of flow cytometry in the array of diagnostic tools for diagnosis of IEI - An overview	Dr. Manisha Madkaikar
10:45 - 11:30	Normal lymphocyte phenotypes - Variations in reactive conditions relevant to clinical practice	Dr. Ananthvikas Jayaram
11:30-11:45	**TEA/COFFEE BREAK**	
	Session 2: 11:45 - 13:30 **Chaired By: Dr. Vijay Kumar & Dr. Shailja Shukla**	
11:45 - 12:30	Panel design for a lymphocyte subset assay in the context of IEI - basic recommendations and best practices	Dr. Sabina Langer
12:30 - 13:00	Case based discussion on interpretation of Lymphocyte Sub-set Analysis in IEI	
13:30-14:00	**LUNCH BREAK**	
Session 3: 14:00 - 16:00 (Wet Lab Demonstration)		
14:00 - 15:00	Case based discussion on interpretation of LSSA in IEI	Dr. Ananthvikas Jayaram
15:00 - 15:30	What happens after flow? Role of genomics & phenocopies	Dr. Manas Kalra
15:30 - 15:45	Report formats - What's it that makes a good report?	Dr. Ananthvikas Jayaram
15:45 - 16:00	**Conclusions & Feedback**	All faculty
16:00-16:30	**TEA**	

Dr. Sindhu Cherian,
University of Washington in Seattle, Washington, USA
Past president of the International Clinical Cytometry Society (ICCS)

Workshop
1 Introduction to MRD – Basics and methods of MRD monitoring

In this session we will review the basics of MRD monitoring in hematopoietic neoplasms with an emphasis in acute leukemia. Benefits and disadvantages of different methods will be discussed with a focus on the role of flow cytometry.

2. **Approach to AML MRD – [Panel design, template formation, internal controls and analysis]**
This session will provide a practical approach to AML MRD assessment using flow cytometry. Topics covered will range from panel design to template development and data analysis. The importance of understanding normal patterns in states of rest and activation will be emphasized and the use of internal controls will be discussed.

Meeting
3. The interplay between genetics and lineage in acute leukemia diagnosis.

In this session the evolution of our understanding of lineage, genetics, and the relationship between the two in the setting of acute leukemia will be explored. The session will review evolving approaches to lineage assignment by flow cytometry using modern classification systems. New categories of acute leukemia and challenge areas in classification where lineage and genetics intersect will be explored using a case base approach.

4. **Getting ready for MRD assessment in the era of immunotherapy**
The approach to therapy in hematopoietic neoplasms has evolved over the past 10 years with an increasing utilization of targeted immunotherapies. In this session we will explore the growing impact of increasing use of targeted therapy in the flow cytometry clinical laboratory and will discuss strategies to optimize residual disease detection in these settings.

Prof Ritu Gupta
All India Institute of Medical Sciences
New Delhi

1 Dissecting Hematological Malignancies: Single Cell Sequencing and Flow Cytometry

Hematological malignancies per se are a good starting point for biological studies at single cell level starting from flow cytometry to single cell sequencing as the cells are already available as a single cell suspension, be it blood or bone marrow.

For diagnosis, prognosis, targeted therapeutics, and disease monitoring, it is important to identify the neoplastic cells, dissect out tumour subclones, study their transcriptome profile and identify drug-resistance clones. To be able to achieve all these, it is important to have insights into the characteristic features of tumour cells that differentiate them from each other as well as from the normal hematopoietic cells present in the tumour milieu.

In this talk, I will discuss how the single cell transcriptomics can be used effectively to study chemoresistance and identify tumour vulnerabilities to develop effective therapeutics.

References:

- Thakral D, Singh VK, **Gupta R*,** Jha N, Khan A, Kaur G, Rai S, Kumar V, Supriya M, Bakhshi S, Seth R. Integrated single-cell transcriptome analysis of CD34 + enriched leukemic stem cells revealed intra- and inter-patient transcriptional heterogeneity in pediatric acute myeloid leukemia. Ann Hematol. 2023;102(1):73-87. doi: 10.1007/s00277-022-05021-4. PMID: 36527458.
- Thakral D, **Gupta R*,** Khan A. Leukemic Stem Cell signatures in Acute Myeloid Leukemia- Targeting the Guardians with Novel approaches. Stem Cell Reviews & Reports 2022. doi: 10.1007/s12015-022-10349-5. PMID: 35412219.

2 Approach to Myeloma MRD – Panel design, template formation, internal controls, and analysis steps

In this lecture, the nuances of MRD evaluation in multiple myeloma with respect to pre-analytical, analytical and post analytical variables will be discussed. The emphasis will be on the salient features of MRD assay for multiple myeloma and its troubleshooting.

Prof Urmi Chatterjee
University of Calcutta

The Ooh, The Aah and The Ouch of - Assay validation of quantitative rare event data acquisition.

Cancer treatment today is faced with major caveats of tumor recurrence and minimal residual disease. Identifying cells responsible for such predicaments focused on elucidating rare events, since cells responsible for relapse are present in extremely small numbers. Cellular abundance is considered rare when the population of interest has a frequency of 0.01% or less. Rare event analysis started more than thirty years ago with counting of fetal red blood cells in maternal circulation. Nowadays, rare event analyses have assumed increasing importance for the diagnosis and monitoring of immunological and hematological disorders. Rare circulating tumor cells or the rise in rare circulating endothelial cells are useful as disease indicators. In addition, the meagre population of cancer stem cells within solid tumors are also evil residents which must be targeted and obliterated along with conventional chemotherapy. Detection and function of such rare cells could give relevant information about the status and stage of disease; however, it is mostly difficult to detect given its frequency. Flow cytometry, capable of analyzing thousands of cells per second, can be extremely useful for rare event detection. Multiparametric flow cytometry of rare cells also allows for simultaneous analysis of additional biomarkers. A tumor cell spiking assay is frequently used to evaluate the sensitivity, specificity, accuracy, and repeatability of the methods or systems for enumeration of rare cells. Detection of such rare events will help detect, characterize and predict precise therapeutic directions for cancer patients in future.

Prof Anubha Gupta
Indraprastha Institute of Information Technology, Delhi,

Cytometry Analysis and Deep Learning

Cytometry, a single-cell technology tool for quantitative measurement and analysis of cellular entities and their constituents, has demonstrated notable advancements in recent years, enabling precise cell analysis at unprecedented scales. This talk endeavors to explore the intriguing intersection of cytometry and deep learning, an emergent field marked by its transformative potential. Since deep learning has the potential to extract complex patterns, this talk will provide insights into how deep learning technology can be harnessed for high-dimensional cytometry data analysis (CDA). Deep learning has significantly improved cytometry data analysis, including automated cell detection and segmentation, feature extraction, as well as classification and phenotype identification. We will present our recent unpublished work on flow cytometry that unveils an innovative approach comprising a multi-head self-attention mechanism-based representation learning, improving the performance of classical unsupervised clustering methods. This work provides an understanding of how newer customized methods can be devised to build state-of-the-art solutions in this domain.

Dr Vainav Patel
National Institute for Research in Reproductive Health, Mumbai

The cellular path to pathogenesis- A tale of 3 viruses

Drawing from the last few years of working with one equilibrium (HCMV) and 2 non-equilibrium viruses (HIV, SARS-CoV-2) cellular immune signatures identified through extensive immune monitoring and correlated with varying disease progression states will be presented for discussion. These include systemic and viral specific correlates for adverse pregnancy outcomes in the context of HCMV congenital transmission, viremic non-progression in the context of HIV-1 infection and immunopathogenesis of SARS-CoV-2. Identification of unifying and putatively actionable signatures suggestive of pan-viral host mediated pathogenesis will be attempted.

Dr Prashant Tembhare
Advanced Centre for Treatment, Research and Education in Cancer, Tata Memorial Center, Mumbai

1 Flow-cytometry approach for diagnosis, classification and monitoring of T-cell CLPD

T-cell/NK cell non-Hodgkin lymphoma (T/NK-NHL) is an uncommon heterogeneous group of diseases. The current WHO classification of T/NK-NHL is mainly based on histopathology and immunohistochemistry. In routine practice, however, the lack of unique histopathological patterns, overlapping cytomorphology, immunophenotypic complexity, inadequate panels, and diverse clinical presentations pose a great challenge. Flow cytometric immunophenotyping (FCI) is a gold standard for the diagnosis, subtyping, and monitoring of many hematological neoplasms. It is a powerful tool for single-cell analysis that allows the study of multiple protein expressions simultaneously in thousands to millions of cells in a short duration of time. The availability of antibodies against a huge variety of markers for cell surface and intracellular proteins, as well as a simple test for T-cell clonality assessment, can provide a far better diagnostic modality for T/NK cell NHL. However, currently, FCI is not used to its greatest potential for the diagnosis and staging of T/NK cell NHL. In this session, we shall discuss the approach for the diagnosis and subtyping of T/NK cell NHL using multicolor flow cytometry assay.

Please also refer to the published paper for additional details. "Tembhare PR, Chatterjee G, Chaturvedi A, et al. Critical Role of Flow Cytometric Immunophenotyping in the Diagnosis, Subtyping, and Staging of T-Cell/NK-Cell Non-Hodgkin's Lymphoma in Real-World Practice: A Study of 232 Cases From a Tertiary Cancer Center in India. Front Oncol. 2022;12:779230."

2 Approach to CLL/Hairy Cell Leukemia MRD: Panel design, template generation, internal controls and analysis.

Measurable residual disease (MRD) assessment has become the standard of care in many hematological neoplasms. Multicolor flow cytometry is a rapid, widely available, and easily applicable highly sensitive technique of MRD monitoring. MRD is a sensitive reflection of disease burden during and after fixed-duration treatment and has been correlated with clinical outcomes. CLL and HCL are both low-grade indolent lymphoid malignancies of elderly patients. These are the only B-cell NHL with distinct disease-defining immunophenotypic signatures, allowing the precise measurement of post-therapy residual disease using multicolor flow cytometry. Typical immune signature of CLL is CD19+, CD20-/+, CD5+, CD22-/+, CD23+, CD43+, CD81+, CD200+, CD45++ and Kappa or Lambda -/+ and that of HCL is CD19++, CD20++, CD11c++, CD22++, CD25+, CD103+, CD123+, CD200++, CD305++, CD45++ and Kappa or Lambda+. Advances in chemotherapeutic regimens and novel therapies have changed the spectrum of diseases and clinical outcomes in these diseases. MRD assessment guides the response to these therapies and allows direct individualized therapeutic strategies, improving the final survival outcomes. In this session, we will discuss the panel design and gating approach for the assessment of MRD in Chronic lymphocytic leukemia (CLL) and Hairy cell leukemia (HCL) using multicolor flow cytometry assays.

The Cytometry Society-India
15th Annual Conference & Workshops
26th -29th October, 2023
Theme: From Basics to Multi-Omics

Prof Sunil Arora
Postgraduate Institute of Medical Education and Research, Chandigarh

Chasing the immune checkpoint molecules in HIV-TB co-infection

Komal Sharma[1], Uzma Pervaiz[1], Aman Sharma[2] and **Sunil K Arora[1,*]**
Departments of [1]Immunopathology and [2]Internal Medicine, PGIMER, Chandigarh, India.
*Email: skarorain@gmail.com

Background: The success of immunotherapy directed at Immune checkpoint molecules (ICMs) in treating various cancers has spurred investigations into whether a similar approach could be effective in addressing specific infections, including human immunodeficiency virus (HIV). Preliminary reports suggest that there may be irregular expression of ICMs in individuals co-infected with HIV and *Mycobacterium tuberculosis* (Mtb). We hypothesize that Mtb modulates the expression of ICMs in co-infected host and targeting the ICMs may lead to innovative strategies for improving the management of these individuals.

Methodology: We conducted a comparative assessment of gene and surface expression of ICMs on T-cells of treatment-naïve individuals recruited in four groups including HIV-1 mono-infected, Mtb mono-infected, HIV-TB co-infected and healthy controls using quantitative real-time-PCR and flow cytometry. Additionally, advanced analysis of whole genome mRNA sequencing (RNAseq) of peripheral blood mononuclear cells (PBMC) from these individuals was performed to identify differentially dysregulated molecular pathways.

Results: The data revealed a significantly increased expression of all five ICMs (TIM-3, CTLA-4, LAG-3, TIGIT, and PD-1) on both the CD4 and CD8 T-cell subsets of HIV-Mtb co-infected individuals in comparison to HIV mono-infected and other groups. The dual expression of these ICMs indicating expansion of 'terminally exhausted' T-cells in co-infected individuals additionally exhibited a positive correlation with viral load with a negative correlation with CD4 T-cell count. The primary pathways identified in the transcriptome profile included Interferon signalling, cytokine signalling, antiviral mechanisms through IFN-stimulated genes, and negative regulation of viral mRNA translation and peptide chain elongation.

Conclusion: These findings underscore the Mtb mediated dysregulated expression of ICMs in HIV-Mtb co-infected individuals and a direct association with faster disease progression. This necessitates further exploration into the possibility of targeting multiple ICMs collectively that may offer adjunct immune-therapeutic options for better management of co-infected individuals.

Dr H Krishnamurthy
National Centre for Biological Sciences,
Tata Institute of Fundamental Research, Bangalore

Role of Follicle-Stimulating Hormone (FSH) in Testicular Development and Spermatogenesis Thereof

Themammalian testis is one of the most prolific among the tissues in the body, with one of its compartments producing hundreds of millions of spermatozoa every day. Thus, it is conservatively estimated that the manufacture of more than five trillion sperm occurs in an incessant manner throughout the adult life of a normal male. This enormously complex and dynamic process of mammalian spermatogenesis is precisely orchestrated by the interplay of endocrine as well as paracrine and intracrine factors. Understanding these processes are extremely important for addressing the questions related to infertility and contraception

Prof Naresh Sachdeva
Graduate Institute of Medical Education and Research (PGIMER), Chandigarh

Unlocking role of proinsulin in type-1 diabetes; from a key autoantigen to a primary therapeutic target

Type-1 Diabetes (T1D) is an autoimmune disease characterized by the destruction of insulin producing beta cells in the islets of Langerhans in the pancreas. In T1D subjects there are few CD8+ T cells in the peripheral circulation that escape thymic selection and start to recognize self-antigens via their diabetogenic T cell receptors. When these cells migrate to the pancreas, they recognize the beta cell-associated epitopes like insulin in peptide centric manner. In a normal individual such TCR-MHC signals are ignored and beta cells survive, however in T1D individuals, the CD8+ T cells release effector molecules like, perforins, granzymes and cause beta cell damage. Proinsulin (PI) is a key autoantigen recognized by CD8+ T cells and in children at risk of developing T1D. In terms of order of appearance and abundance as well, majority of T cell epitopes are located in the PI region. However, due to low frequencies of circulating autoreactive T-cells it is always a challenge to detect T cells recognizing PI-derived epitopes. However, with the introduction of MHC multimers, especially, MHC dextramers, it is now feasible to detect the epitope-specific T cells by flowcytometry. Further, advancements in multiparametric flowcytometry has enabled functional analysis of these cells. Our group has developed an *in vitro* approach for enrichment of preproinsulin (PPI)-specific T cells from T1D subjects for improved detection and functional analysis of autoreactive T cells using MHC dextramers.

Next, combining the approaches of identification of epitope-specific regulatory T cells (Tregs) using MHC multimers and Treg specific markers can facilitate the scope of antigen-specific immunotherapy in humans. Animal studies have indeed shown that islet antigen-specific Tregs can reverse T1D by targeting T cells that infiltrate pancreas. We have generated PPI-specific Tregs from natural and induced Tregs and assessing their specificity, stability and suppressive ability to inhibit beta cell-specific effector T cells. Finally, we assessed the potential of cord blood (CB) in generation of PI-specific Tregs by using HLA class II tetramers. For this, CD4+CD25+CD127^{low} and CD4+CD25-T cells derived from CB were cultured in the presence of PI derived peptides, TGF-β and rapamycin. PI-specific Tregs were then selected using allele-specific HLA II tetramers loaded with PI derived peptides. In co-culture experiments, the PI-specific Tregs suppressed the proliferation of effector T cells. Thus, PI can be utilised generation of functional antigen-specific Tregs for immunotherapeutic approaches in T1D subjects.

Dr Anugya Bhatt
Chitra Tirunal Institute for Medical Sciences and Technology, Trivandrum

Mesenchymal Stem Cells-Platelet interactions & wound healing

Wound healing is a complex process involving different phases. In chronic wound condition, the healing process is delayed or fails to repair the damaged tissues resulting in ulceration and complete destruction of wound area. Cellular therapies are emerging using Mesenchymal stem cells (MSCs) due to their non immune nature. Primarily wound healing process is lead by the cellular secretomes and cellular secretome largely depends on the cellular interactions. MSCs release several immunomodulatory proteins, cytokines, growth factors, signaling molecules called secretome, and have the potential to accelerate the wound healing process.Platelets are anucleated cells essential for wound healing in all phases. In the present study we tried to explore the interaction of MSCs and platelets, and the effect of platelet induced MSCs secretome on wound healing process. We have shown the cellular interactions. It was observed that platelet induced MSCs secretome enhances wound healing significantly even in impaired wound healing conditions like diabetes and hypoxia.

Dr Paresh Jain
BD Biosciences, Central and South Asia and Japan

Translational approach for assay menu expansion in a clinical flow cytometry lab

In this talk, I will cover the journey of a clinical flow assay starting from conceptualization of its potential clinical use, through its assessment for feasibility and analytical validation, to its evaluation for performance and utility in the clinical setting. Then, I will share a framework to map the 'maturity and readiness' of potential clinical flow assays across this translational journey using suitable examples from a diverse range of hematological and immunological applications.

Dr Kunal Sehgal
SEHGAL PATH LAB
Mumbai

1 Immunophenotype variants of normal populations- Circumventing pitfalls in MRD reporting.

This talk will highlight the importance of knowing the phenotype of normals especially in relation to high event MRD analysis. Various normal populations seen in the background of disease or as part of regenerating marrows in different MRD assays will be discussed. Immunophenotype of few normal populations which may show phenotypes overlapping with MRD populations and how to identify these using existing markers in the panel will be discussed. Examples and plots of common phenotypic variations post therapy will also be shared.

2 MRD Report format - What's it that makes a good report?
In this talk we will discuss salient features that should be included in a MRD report.

Important parameters to be included in reports as follows
Total Viable events
MRD events
Denominator used
Sensitivity of assay including LOD, LOQ
Diagnostic immunophenotype
Description of the MRD population and related parameters
Viability in case of transported samples
MRD Thresholds for making clinical decision

Reference to current guidelines and common practice points will be shared.

Dr Rutvi G Dave
CMC, Vellore

Going beyond conventional flow cytometric testing - Platelet Activation Tests (PACT)

Platelets play an important role in the formation of a primary haemostatic plug that stops bleeding at the site of injury. Platelet function defects (PFDs) may be inherited or acquired and manifest as mucocutaneous bleeding of varying severity. Patients with PFDs may have normal platelet count or thrombocytopenia. Timely diagnosis of PFD is important to enable risk stratification of patients and offer appropriate therapeutic intervention at the time of bleeding. PFDs are heterogeneous in pathogenesis and may result from abnormalities in the platelet glycoproteins, receptors for soluble agonists, content/release of granules, transcription factors, signal transduction pathways, or membrane phospholipids. Light Transmission Aggregometry (LTA) has been the Gold Standard for the diagnosis of PFDs since decades. LTA requires preparation of platelet-rich plasma (PRP) and measures the changes in transmission of light through PRP during agonist stimulation. Although the pattern of findings in LTA for various known PFDs has been well characterized, it still lacks standardization in worldwide surveys. LTA has several limitations. It is labour-intensive and lacks sensitivity for mild PFDs including storage pool disorders. The requirement of a large blood volume and preferably fasting sample makes LTA a less feasible test for pediatric and especially neonatal patients. Interpretation of LTA may be inconclusive if the PRP platelet count is less than 150 x 10^9 /L, hence it cannot be used to assess platelet function in the setting of thrombocytopenia. Moreover, LTA requires a dedicated and customized aggregometer which is usually available only in highly specialized diagnostic laboratories and as a result, PFDs remain underdiagnosed. Since LTA is affected by numerous pre-analytical variables, testing needs to be completed on fresh samples within 4 hours of sample collection, and hence cannot be performed on stored/transported samples received by the referral equipped laboratories. Flow cytometry has emerged as an alternative technique for assessing platelet function. The International Society of Thrombosis and Haemostasis (ISTH)-Scientific and Standardization Committee (SSC) also recommends the use of flow cytometry in the diagnostic work-up of PFDs. Flow cytometry-based platelet activation test (PACT) has major advantages over LTA since it requires a very small volume of whole blood, no need for centrifugation or a fasting sample and it can be interpreted irrespective of thrombocytopenia. This makes it a convenient test for pediatric patients and thrombocytopenia cases. Furthermore, it does not require a customized and dedicated aggregometer and can be performed even on a basic four-color flow cytometer which is increasingly available in numerous diagnostic laboratories for various applications. PACT allowed simultaneous measurement of a broad range of different markers of platelet function thereby allowing for a more comprehensive analysis of various pathways involved in platelet function. The expression of various activation markers like PAC-1, P-selectin (CD62P) and CD63 can be measured after activation of platelets with various agonists to explore the integrity of common platelet signalling pathways. The platelet signalling pathways that can be explored using various platelet agonists include:

- the collagen pathway, stimulated through GPVI using Convulxin / Collagen Rich Peptide which mimics the action of Collagen.
- the thrombin pathway using the Thrombin Receptor Activating Peptide-6 (TRAP)
- the ADP pathway using ADP
- the Thromboxane pathway using TXA2
- Platelet GpIB-VWF binding was assessed using Ristocetin

The expression of activation markers like PAC-1, P-selectin (CD62P) and CD63 are measured after activation with the above mentioned agonists. PAC-1 antibody detects the conformational change in the receptor αIIb-β3 upon platelet activation which is necessary for fibrinogen binding and platelet aggregation. When platelets get activated by agonists, CD62P is translocated to the platelet surface membrane upon α-granules release, and CD63 is translocated to the platelet surface membrane upon dense release. Ristocetin-induced Von Willebrand factor (VWF)-platelet binding can also be measured using Anti-VWF antibodies.

Thus, PACT overcomes all the limitations of LTA and enables more comprehensive assessment of various platelet activation pathways in a physiological environment without any cell loss or pre-activation.

Dr Sushil K Ramadasi
Beckman Coulter Life Sciences

Single Cell Sorting.

Single cell sorting plays crucial role in single cell isolation leading to further culture of clones, genomics or proteomics of transformed cells. Flow cytometry-based cell sorting overweighs advantages of conventional methods of cell sorting. Flow cytometry science is developing with benefits of advanced detection sensitivity, advanced features and smaller in size. In the present session we will discuss around what is single cell sorting, why single cell.

Prof Dragan Jevremovic
Mayo Clinic, Rochester, MN, USA

PCPRO and Myeloma-MRD screening.

Detection of minimal residual disease (MRD), by next-generation flow cytometry (NGF) and/or next-generation sequencing (NGS), has become a routine test in the survival prediction and management of patients with treated multiple myeloma (MM). The value of MRD detection is mostly shown in patients with complete response to therapy (CR). However, in practice, clinical response assessment is often incomplete at the time of bone marrow (BM) evaluation. Pathologists must rely on morphological assessment of the BM aspirate to decide whether to pursue MRD testing. As a result, there is a large proportion of patients for whom MRD testing reveals a PC clone of significant size, far beyond the MRD test's purpose. In this study we examined the utility of screening flow cytometry immunophenotyping in assessing the need for MRD testing in a cohort of patients treated for MM and plasma cell leukemia (PCL). Our method of screening was the plasma cell proliferation assay (PCPRO) which measures DNA content of the clonal plasma cells and can detect the presence of the aberrant clone with sensitivity of approximately $4.4x10^{-5}$. We show that PCPRO is an effective screening assay for MRD testing, which saves resources for the laboratory, including antibodies and, most importantly, analysis time.

Dr Ranjeet Sahoo
All India Institute of Medical Sciences, New Delhi

Importance of MRD in hematological malignancies

Minimal or measurable Residual Disease (MRD) assessment has emerged as a critical tool in the management of hematological malignancies. Hematological malignancies, including leukemia, lymphoma, and myeloma, are characterized by abnormal proliferation of blood cells, often requiring intensive treatment approaches. MRD refers to the small number of cancer cells that may persist in a patient's body after therapy, but are undetectable by conventional methods. The evaluation of MRD provides invaluable information about treatment response and disease progression.

This abstract explores the significance of MRD assessment in hematological malignancies. MRD testing techniques have become increasingly sensitive, allowing for the early detection of residual disease. Identifying MRD positivity is associated with an increased risk of relapse, helping clinicians make informed decisions regarding treatment intensification or maintenance therapies. Conversely, MRD negativity often signifies a favorable prognosis.

The application of MRD assessment varies across different hematological malignancies, with notable success in acute lymphoblastic leukemia and multiple myeloma. Ongoing research aims to standardize MRD assessment methods and expand their use in clinical practice. In summary, MRD assessment has transformed the management of hematological malignancies, offering a precise, real-time evaluation of treatment response, aiding in risk stratification, and guiding therapeutic decisions to improve patient outcomes.

Dr Karthik Bommannan
Cancer Institute (W.I.A.), Adyar,
Chennai

Approach to B ALL MRD: Panel design, template generation, internal controls & analysis

Acute leukemias are common hematologic malignancies in both adult and pediatric age groups. Among lymphoid lineage acute leukemias, precursor B-lineage acute lymphoblastic leukemia (B-ALL) is the most common entity. Before treatment, patients diagnosed with B-ALL are risk-stratified based on their age at diagnosis, gender, leukocyte count, cytogenetics, and molecular genetic parameters. This baseline risk stratification is helpful for provisional prognostication and early treatment decisions (e.g. Imatinib in *BCR-ABL1* positive B-ALL). As the underlying biology of malignant cells is ever under exploration, these commonly followed baseline risk stratification parameters are not always foolproof in predicting treatment response.

During treatment, the rapidity and extent of leukemic blast cell clearance in bone marrow (BM) is a valuable indicator of treatment responsiveness. During any phase of treatment, the presence of <5% blasts in the BM sample assessed on Romanowsky stained smears is considered as complete remission (CR) of the disease. However, morphologic assessment has major limitations. Being subjective, the technique cannot differentiate between proliferating hematogones, regenerating myeloid blasts, reactive lymphocytes, and leukemic blasts. Even in patients with morphologic CR, there still might be a substantial load of sub-microscopic leukemic cells in the BM, resulting in relapse. This sub-microscopic level of residual leukemic cells constitutes minimal/measurable residual disease (MRD). Currently, MRD is an independent predictor of relapse and is not influenced by the cytogenetic and molecular genetic profile of the disease. MRD-positive status at any time point of treatment is associated with an increased risk of relapse. The most immediate application of MRD testing is the identification of patients who are candidates for early treatment intensification because it is always better to give treatment intensification at an early stage where the leukemic cell burden is 10 to 100 times lower than the later stage of full-blown relapse.

MRD can be reliably assessed by both molecular and flow cytometry (FCM) techniques. Among these techniques, FCM is the commonest modality used as it is readily available, relatively easy to standardize, reproducible, and has a quick turnaround time to aid rapid clinical decisions. As of today, an MRD level of ≥0.01% in BM is of clinical relevance in patients being treated for B-ALL. Using bulk processing techniques and ≥10 color FCM MRD panels, it is feasible to detect as low as 0.001% residual leukemic cells in the bone marrow.

In the current workshop, we will be discussing the concept of B-ALL MRD analysis by FCM. The areas covered will be optimal sample collection, processing techniques, assay standardization, basic gating strategies, and common pitfalls encountered during analysis.

Dr G Smeeta
All India Institute of Medical Sciences, New Delhi

Dr Nupur Das
All India Institute of Medical Sciences, New Delhi

B-ALL MRD – Concepts & Case Studies

Minimal/measurable residual disease (MRD) refers to a chemotherapy/radiotherapy-surviving leukemia cell population diagnosed through phenotypic marker patterns or differential gene patterns through analysis by flow cytometry (FCM), polymerase chain reaction (PCR), or next-generation sequencing (NGS). The primary clinical purpose of monitoring MRD is to determine the response to therapy, and guides post-remission treatment strategies and predicting impending leukemia relapse. There are two strategies for flow cytometric MRD detection: "Leukemia-Associated Immunophenotype" (LAIP) and "Difference from Normal" (DfN). LAIP is identified at diagnosis by comparing the antigen expression profile of leukemic cells to their reference cell counterparts and DfN is defined by recognizing immunophenotypic deviation from their normal counterpart population (hematogones) through the evaluation of antigen expression patterns. Baseline phenotype is required in less than 10% of B-acute lymphoblastic leukemia (B-ALL), specifically those with leukemic blasts with overlapping hematogone patterns and/or dim to negative expression of CD10. Hence, a combination of DFN and LAIP approach is recommended for reliable MRD assessment.

The goal of our presentation is to provide a practical approach along with common diagnostic challenges in B-ALL MRD assessment using flow cytometry by giving examples from clinical case scenarios. The gating strategy includes the removal of doublets, debris and gating of mononuclear cells. Further CD19-positive cells are gated and evaluated for different patterns to detect residual leukemic cells. Common markers used for analysis in B-ALL MRD include CD45, CD34, CD19, CD10, CD20, CD22, HLADR, CD38, CD81, CD58, CD123, CD73 and CD304. Cases of both CALLA positive and CALLA negative B ALL MRD will be discussed with different gating strategies. Cases with hematogone hyperplasia which may overlap and partially obscure the residual blasts will also be discussed, which require meticulous assessment of multiple bivariate plots. Cases with multiple clones of residual leukemic blasts after therapy is also discussed emphasizing on their distinction from hematogones. Furthermore, following anti-CD19 therapies, residual leukemic cells may have a CD19-negative immunophenotype and therefore, false MRD negative may be detected. In these cases, alternative B cell gating markers, including the CD22 and CD24 are used to detect residual leukemic cells. Cases of phenotypic shift with differential expression of phenotypic markers between the diagnostic and MRD/relapse samples may also occur, which should be considered while evaluating MRD.

Dr Jasmita Dass
All India Institute of Medical Sciences, New Delhi

Approach to T ALL MRD: Panel design, template generation, internal controls & analysis

Measurable residual disease (MRD) was first assessed in Precursor T-Acute lymphoblastic leukemia (T-ALL). It was assessed based on the expression of TdT in cells with T-cell markers with the premise that the normal peripheral blood (PB) or the bone marrow (BM) do not have any normal counterparts with this phenotype. The other property that was exploited was the dim to negative expression of of surface CD3 expression in T-lymphoblasts. The kinetics of MRD decline in T-ALL are different from B-ALL with respect to slower decline of MRD at end of induction (EOI) time-point when compared to End of consolidation (EOC) time-point. The EOC time-point is more informative to guide treatment decisions than the EOI time-point. Also, the MRD positivity in T-ALL is also predictive of the extramedullary relapses in addition to intramedullary relapses. The cut-off for a negative MRD is 0.01% consistently. However, at end of induction, a level of >1% should be taken as high risk and for end of consolidation, a cut-off of >0.1% identified patients at a high risk of relapse. Recent data indicates that EOI MRD status correlates with RFS and OS. BM is usually used as the starting material for MRD assessment. In T-ALL the PB MRD is only about half-a log less than the BM and correlates well with BM MRD unlike B-ALL. However, still, BM is used for MRD assessment considering that patients usually undergo BM examination at the EOI and EOC time-points. As with all MRD estimations, first-pull BM samples are submitted for MRD assessment. Panel design: T-cell maturation starts with a CD34+ T-cell precursor that migrates to thymus. Immature thymic precursors express CD99 and rapidly gain TdT expression and lose CD34. CD10 is expressed for a short period during subsequent maturation. There is a gradual loss of CD99 upon maturation. Blasts in T-ALL show a maturation arrest or express aberrant antigens that can be used for their identification. The initial panels used in T-ALL relied on the principle of detecting immaturity associated markers such as TdT and CD99 in combination with a mature T-cell marker like CD5/ CD7/ CD2/ cytoCD3 or a population of cytoCD3+/sCD3- cells. However, T-ALL cases deemed MRD+ showed a loss of TdT, CD99 and CD1a expression that was present in these cases at baseline in >50% blasts. Therefore, the approach to T-ALL MRD has changed. The gating markers for T-ALL MRD include CD7, cytoplasmic CD3 (clone UCHT1), surface CD3 in an alternative clone, CD45 (also a MRD marker when under-expressed) along with CD56 and CD16 either on the same fluorochrome or on different fluorochromes dependent on the flow cytometer available. It is primarily centred around gating all CD7 positive cells followed by selection of cytoplasmic CD3+/surface CD3- or dim cells. This population also contains the NK cells which are excluded either upfront or at the end of analysis based on the CD56/CD16 expression. If the exclusion is done upfront care should be taken to not exclude the CD56+/CD16+ T-ALL cases. After gating the CD7+/sCD3- or dim/cyCD3+ cells, the population will contain MRD in positive samples, NK cells, immature NK cells and mature T-cells with lack of CD3(possibly on account of cytoplasmic processing). For most of the downstream analysis, CD5 vs CD38 or CD5 vs CD56/16 gating can be utilised and all populations gated can be further assessed for the expression of CD4 and CD8. Mature T-cells with lack of CD3 show a split into CD4+ and CD8+ population. Immature NK cells can be further discriminated using the property that they are CD45 bright, CD38 positive and show a negative to weak expression of CD8 but are negative for CD5 and CD4. In addition, the mature NK cells are segregated based on the CD56/CD16 expression along with bright CD45. Suspect MRD populations are further assessed based on the CD4 vs CD8 plot with either a predominant expression of only one of the markers or double positive or a double negative population. In addition, the suspect MRD population is assessed for the leukemia associated immunophenotype (LAIP) present at baseline such as a dim CD5/strong CD5/ dim CD45/ very bright CD7 expression etc. If using a T-colour panel or more, CD48 can also be added. CD48 is expressed by normal T-cells and its expression is reduced in T-ALL cases.

Dr Kahliq-ur-Rahman
SGPGIMS, Lucknow

Myeloma MRD: Concept & case studies

The disease activity in MM can be quantified using several modalities like biochemical analysis (M protein, light chain assay), bone marrow examination, imaging modalities like MRI (Magnetic Resonance Imaging) & PET. Considering the heterogeneity and the patchy involvement of this disease, these diagnostic modalities may not be having sufficient sensitivity to detect exceptionally low level of disease. PCR, NGS and MFC are the suitable technologies to detect such a lower level of residual disease (MRD). NGS & PCR are technically more challenging, need lots of bioinformatic support and have a higher turnaround time. Hence, MFC appears to be the most suitable technique for the MRD assessment.

Dr Tina Dadu
BLK MAX Super Speciality Hospital, New Delhi

AML MRD: Concept and case studies.

Minimal Residual Disease (MRD) is an important biomarker in optimum care of patients with Acute myeloid leukemia (AML) that is used for prognostic, predictive, monitoring, and efficacy-response assessments.

Assessment of MRD in acute myeloid leukemia (AML) is challenging. Several technologies are available for MRD quantification, but the assays and reporting lack standardization and comparability. Still, detection of MRD by any methodology during morphological remission after standard chemotherapy is a strong prognostic factor for subsequent relapse and shorter survival in patients with AML.

The goal of our presentation today is to make AML MRD by flow cytometry as objective as possible, with a and step by step approach. Approach would include gating all mononuclear cells, and then exclude the normal progenitors one by one, which will leave us with fewer populations and make it easier for abnormal populations to get highlighted. The approach includes both diagnostic leukemia-associated immunophenotype (LAIP) and different from normal (DfN) aberrant immunophenotype to enable tracking of diagnostic and emergent leukemic clones. Markers used for analysis include CD34, CD117, CD45, HLADR, CD38, CD123, CD64, CD36,CD33, CD13, CD56, CD7, and HLA-DR.

Prof Sanjeev K Gupta
All India Institute of Medical Sciences, New Delhi

Challenges in MPAL through case studies.

"Mixed Phenotype Acute Leukemia (MPAL)" is a subtype of 'Acute Leukemias of Ambiguous Lineage (ALAL)', where blasts express antigens of more than one lineage to such a degree that it is not possible to assign the leukemia to any one lineage with certainty. MPAL/ALAL can be categorized into two categories, "MPAL/ALAL with defining genetic abnormalities" (e.g. BCR-ABL1, KMT2A rearrangement, ZNF384 rearrangement etc.) and "MPAL/ALAL, immunophenotypically defined" (B/Myeloid, T/Myeloid, rare types like B/T etc.). MPALs can contain distinct blast populations each of a different lineage (bi-lineage), or one population with multiple antigens of different lineages on the same cells (bi-phenotypic), or a combination. In the latter setting of a single blast population, the criteria for lineage assignment are more stringent, and there are several caveats as to how the expression of lineage-specific markers of Myeloid, T- and B- cell lineages should be interpreted to achieve a diagnosis of MPAL. In the recent update of the WHO classification of Haematolymphoid Tumours (2022), the lineage assignment criteria for MPAL are further refined to emphasize principles of intensity and pattern.

Dr Sachin Jain
Fortis Memorial Research institute
Gurgaon

MRD analysis : Euroflow perspective, Infinicyt Software

This session will highlight use of Euroflow based protocols and Infinicyt software in high sensitivity MRD analysis.

Salient features of infinicyt software as follows:

File Merge: allows to combine several files into one file to perform global analysis and straightforward data interpretation. This tool helps in analysing simultaneously more than 5 million events acquired in multiple tubes for highly sensitive MRD assays.

Automatic Population Separator (APS) Tool: APS Diagram, based on the Principal Component Analysis, maximizes the potential of antibody panels by immunophenotype-based automatic separation of cell clusters. The APS takes all parameters into account for the best possible separation of events, providing information about parameter significance for this segregation.

Clustering Tool: automatically connects and relates all events of a sample, dividing them into groups (clusters) according to their relative distance in the multidimensional space.

Reference Image Tool: This tool whereby images and statistics from diagnostic sample can be stored and loaded for future comparison in MRD analysis samples.

Offline Compensation: User can perform an automatic (offline) compensation using compensation controls including unstained sample.

Automated Gating & identification of population: AG&I tool provides the identification of all cell populations in raw files by comparison against EuroFlow reference databases. The software also suggests an identification for cell populations that do not completely match the database, allowing to a faster review of all cells in a sample. For eg; **AG&I MM-MRD database** is meant to be used for the automated gating and identification (and also reporting) of all cell populations present in the bone marrow samples from PCD treated patients. Database contains references of normal phenotypes from bone marrow samples stained with the MM MRD panel. All clusters of events from an .fcs file stained with the MM-MRD combination are compared with the normal reference database populations and classified either unequivocally as a normal population, or as a population to be closely reviewed by the expert.

Prof Kalpana Luthra
All India Institute of Medical Sciences, New Delhi

Unlocking the power of cell shorting

Since the invention of the first prototype of a Fluorescence Activated Cell Sorter, the technology has become a powerful tool to analyse and sort individual cells based on their functional status. Flow cytometry provides a robust statistics of thousands of individual cells and can detect rare events at a frequency below 10^{-4} cells. This feature is especially important for biotechnological applications, because it is usually the rare cell which is of interest: the cell with altered properties, a higher production rate, better metabolic parameters or containing the protein with a higher binding affinity. Such rare cells with altered properties are the main target of cell sorting. Flow cytometric cell sorting is a versatile and well-established research tool to isolate cells in high purity and speed. Multiparameter sorting permits the identification and isolation of specific cell subpopulations based on qualitative and/or quantitative antigen expression as well as on positive and negative selection criteria within a single selection procedure. With the advent of numerous cell therapy approaches for clinical applications, mainly driven by the success of chimeric-antigen-receptor-modified (CAR) T cells, interest in the clinical use of flow cytometric sorting is rising. To be able to sort, it is a prerequisite that flow cytometric methods have been established that allow the characterization of a specific cellular property, by sorting cells from different observed subpopulations. Subsequently, these cells are analyzed by other methods, thus linking different types of information and analytical methods to enhance the understanding of cell behavior. Many biological samples are cell mixtures, and it was shown that sorting the different cell types of model cell mixtures and of cord blood prior to microarray analysis revealed gene expression patterns (transcriptomics) that were otherwise hidden. Biotechnological applications can be grouped into either the screening for specific features of biomolecules, mainly proteins, or the screening for cells with specific superior features. Sorting of cells upstream of single-cell RNAseq library preparation allows to enrich for singlet cells (essential for any single-cell method) and if needed, to enrich for subpopulations of interest. These subpopulations may be viable cells, non-apoptotic cells, cells in a specific cell cycle phase, or cells expressing a sortable marker to enrich for specific or rare cell types. Recently, it has been shown that cell numbers are important for the standardization and normalization of 16S rRNA gene sequencing data. Flow cytometric applications achieving high resolution of light scatter and DNA content discrimination can be used to characterize and resolve complex microbial community structures, such as environmental microbiota or intestinal microbiota by generating community patterns. In our laboratory, we performed antigen specific single cell sorting of single B cells from infected children, with elite plasma neutralizing activity. We used a labelled native like HIV-1 envelope trimer as the antigenic bait for the sorting. Monoclonal antibodies from the sorted single cells were generated and characterised for their ability to neutralize HIV-1 clade C viruses, to serve as potential anti-HIV-1 reagents.

Prof Narinder K Mehra
President Indian Society of Histocompatibility & Immunogenetics (ISHI)
Founder Head, Dept of Transplant Immunology & Immunogenetics
All India Institute of Medical Sciences, New Delhi

Evaluation of Transplant Immunology

Organ Transplantation is a medical procedure in which the damaged or diseased organ of a recipient is replaced by a healthy one. Successful organs for transplantation include Kidneys, heart, liver, lung and pancreas; nonetheless for the graft to have long-term acceptance, it is important to overcome the immunological barrier, both for avoiding graft rejection as well as for clinical management. In this context, there are two challenges, namely HLA mismatching between the donor and recipient, and the problem of antibody mediated rejection (ABMR). It is important to characterize the Preformed and *de novo* development of donor specific antibodies (dnDSA) that are often responsible for causing graft rejection following solid organ transplantation. The spectrum of antibody mediated insult ranges from hyperacute rejection to chronic antibody mediated rejection (AMR) leading ultimately to graft loss. The development of bead based technologies allows the detection and characterization of HLA antibodies with high degree of sensitivity and specificity, highlighting the relative importance of ABMR as the major player responsible for graft loss and affecting over 60% of late kidney grafts. While anti-HLA DSAs have been widely associated with poor graft survival, the role of non-HLA antibodies, particularly those directed against endothelial cells, is beginning to be appreciated. Of these, MICA (Major Histocompatibility Complex class I chain related molecule A) antibodies are the most notable because of their potential in promoting hyper acute rejection and/ or exaggerated ABMR. MICA is a potent activator of NK cells via their receptor NKG2D leading to activation of the NK cell mediated arm of allograft rejection. A comparative analysis of Luminex based antibody MFI values with the CDC and FCXM results revealed that a cut off MFI value of 3000 for Luminex SAB-based assay is significantly correlated with the FCXM positivity while a value of 7000 and above predicted a positive CDC cross match. These cut-off values could help in determining donor specific and anti-HLA antibodies on a Luminex platform, as a surrogate marker for CDC and FCXM test results and help in resolving the limitations of different cell-based techniques. In recent years, the ddcfDNA has emerged as a promising non-invasive diagnostic and monitoring tool for the early detection of ABMR, monitoring response to treatment as well as identifying a proportion of immune quiescent patients who may benefit from immunosuppression minimization.

Dr Aseem Tiwari
Transfusion Medicine, Medanta-Gurugram

CDC crossmatch: Principle, methodology, analysis, interpretation, modification & pitfalls

Compatibility testing between renal transplant recipients and their potential donors is an essential component of the pre-transplant work-up. Compatibility tests are used to detect donor-specific antibodies (DSA) in the recipient and the absence (low-risk) or presence (high-risk) of DSA helps in deciding the risk of antibody-mediated rejection (AMR) after renal (or other solid organ) transplants. The CDC crossmatch is not only the first but possibly the most widely used compatibility test, even today, for identifying DSA.

The CDC crossmatch (CDC-XM) combines recipient serum and donor cells. If donor-reactive allo-antibodies are present in the patient's serum, these antibodies can bind with donor cells. Antibody-antigen complexes, in turn, can activate the complement cascade (complement is added to the test as a reagent), leading to complement-mediated cytotoxicity. The resulting cell death (cytotoxicity) is analyzed by a trained observer, using an inverted microscope. Certain modifications like the addition of anti-human globulin (AHG) or the use of Dithiothreitol (DTT) to the CDCXM have led to improved sensitivity of CDC-XM as compared to the conventional CDC-XM.

Although the CDC-XM has remained the mainstay test in the practice of renal transplantation, the current need for routine CDC-XM is less clear with the advent of more sensitive techniques. There are now many more options available for determining the likelihood of donor-specific antibody-mediated responses including flow crossmatching (FCXM) and Luminex crossmatching (LXM). Several centers have also adopted “virtual crossmatch,” in addition to physical crossmatching. In virtual crossmatch the Luminex single antigen bead (SAB) is used to identify anti-HLA antibodies in the recipient, which is then virtually matched against the donor’s HLA typing.

Dr Gurvinder Kaur
All India Institute of Medical Sciences, New Delhi

CDC crossmatch: Concept & case studies

Presence of alloimmune antibodies in transplant recipients especially against donor HLA antigens correlates significantly with an increased risk of hyperacute rejection. Alloantibodies may be present pre-formed or may be generated *de novo* post organ or post HSCT. Accurate identification and regular monitoring of donor specific antibodies (DSA) in pre and post transplant patients is therefore crucial in donor-recipient matching and preventing graft loss. Complement dependent cytotoxicity (CDC) crossmatching was developed by Paul Terasaki in 1964 and continues to be an important part of clinical decision making in transplant clinics. Modifications of CDC Crossmatch AHG-CDC XM, DTT-CDC XM have been developed to augment CDC XM sensitivities and reduce false positives which will be discussed considering clinical case studies.

Flow crossmatch: Case studies

HLA disparities among donor recipient pairs can give rise to pathologic DSAs and associate with poor graft outcome. Flow crossmatch (FXM) allows detection of complement fixing and non complement fixing DSAs against HLA and non HLA antigens with higher sensitivities than CDCXM. Careful interpretation of CDCXM, Luminex XM, T or B cell FXM and virtual XM is needed for predicting humoral alloimmune potential of matched donor recipient pairs and favourable graft outcomes. Examples of DSAs against HLA and against non HLA antigens such as MICA will be discussed and relative advantages/ disadvantages of different types of cross matches will be illustrated.

Dr Hemant Agrawal
Flowcytometry Solutions Pvt. Ltd.

Flow Cytometry data analysis and presentation

Fluorescent Activated Cell Sorting (FACS) or Flow Cytometry is widely used in basic and clinical research. To harness the real power of this state of art technique, accurate data analysis of FACS data plays a major role. Correct data analysis and presentation is not only important but also critical to adequately identify or functionally characterize the multiple populations of interest within the immune system. In the last few years, the number of parameters (colors) simultaneously used in flow cytometry experiments has increased. This is enabled by the availability of high-end instruments and powerful data analysis tools. In this lecture, we will cover analysis of single or multicolor flow cytometry data and guidelines for the correct data analysis and presentation. Participants will learn to open their FCS files with the data analysis software, explore their data using various display options, perform gating, and quickly generate graphical and statistical reports to identify various population of interest. Participants will also be provided with the knowledge to understand potential technical issues with the data. In sum, this lecture will help the researchers to understand the importance of correct flow data analysis and presentation.

Dr Rajesh Pandey
CSIR-Institute of Genomics and Integrative Biology (IGIB), Delhi

Single Cell Omics facilitating canonical and non-canonical understanding of the host immune system

Single Cell Genomics has given us the granularity required to explore, elucidate and understand the dynamics of immune response, like never before. It has facilitated understanding at the level of cell types and their role in immune response for diversity of diseases. Post COVID-19, this assumes significant importance as we prepare for Future Pandemic Preparedness. In this journey, it is incumbent on us as to what additional information can be extracted from the Single Cell Genomics data, which are important modulators for disease severity. Some of the key non-canonical aspects which can be explore are, i) functional role of Noncoding RNA, ii) journey from cell type to individual cells, iii) dynamics between whole transcriptome analysis (WTA), Antibody-sequencing & TCR/BCR, iv) can we detect ingested microbes within the cells? Moving forward in this journey, research community (collectively) should be making efforts towards these questions which are pertinent for infectious diseases.

Prof Arindam Maitra
National Institute of Biomedical Genomics, West Bengal

Our Journey from Bulk to Single-cell Transcriptomics in Oral Cancer: Novel insights and new Horizons

Tumors are complex ecosystems composed of diverse cell types, including malignant, immune and stromal subsets. Interplay between these diverse cell types are the major driver for initiation, progress and response of tumour. Our recent bulk tumor sequencing studies have led to identification of some of the genomic, epigenomic and transcriptomic alterations which drive tumorigenesis in gingivobuccal oral cancer (OSCC-GB), the most predominant cancer of men in India, but information on heterogeneity of cell types and cell states within the tumours is yet to be explored.

To address this question, we undertook single cell transcriptome profiling of tumor biopsies of treatment naïve OSCC-GB patients. We identified cell types present in the tumor ecosystem and have inferred the dominant gene expression programs of the malignant and non-malignant cells, classified by a novel method developed in house. We identified unique malignant and non-malignant cell types and states which might have important impact on tumour initiation and progress. Our results provide novel information on heterogeneity of gene expression in OSCC-GB which might lead to improved understanding of cell type diversity and cellular transitions in oral cancer.

Prof Sabina Langer
Sir Gangaram Hospital, New Delhi

Case based discussion on interpretation of lymphocyte sub-set analysis in IEI

Panel design for lymphocytes subset assay in the context of Inborn errors of immunitu(IEI) –basic recommendations and best practices.

The basic lymphocyte subset along with immunoglobulin levels, clinical work up form a robust tool which can help pick up 60 to 70% cases of IEI. The basic lymphocyte subset must include markers for T cels (CD3,panT cell marker), B cell(CD19) ,NK cells (CD56+CD3-) along with helper T cells (CD4+CD3+,Th) and cytotoxic T cells (CD8+CD3+,Tc). This basic lymphocyte subset analysis may require to be supplemented by further T cell and B cell extended panels in certain situations which include less severe combined immunodeficiency(CID),leaky SCID where mother's memory cells crossover and falsely increase the absolute counts in the infant. The extended T cell subset includes naive T cells, Memory T cells (effector memory, central memory),terminaly differentiated effector memory cells. The extended B cell subset analysis includes pre germinal centre or naïve B cell,post germinal centre or memory B cells ,class switched memory B cells,non-switched /marginal zone memory B cells and IgM only memory B cells. The TCRYδ positive and negative T cell susbsets and HLADR expression on T and B cells .(table1)

There are various practices across the globe for diagnosis of inborn errors of immunity. These include EUROFLOW consortium which has a 8 color 14 antibodies to screen for Primary immune deficiency disorders. The Lymphocyte subset analysis can be done through dual platform (the counts are calculated using hematology analyser) or single platform (using beads). Using single platform technique with preformed gates can formulate an easy and rapid technique for fast and easy reproducibility of lymphocyte subset.Some of the examples of readymade tubes available include lyophilized tube for primary immune deficiency screening and duraclone tubes for extended B cells, T cells and T regulatory cells.In house cocktails can also be made for screening Primary immune deficiency diseases. The further immunophenotyping is available in the referral centers based on the findings of basic lymphocyte subset analysis for severe combined immunodeficiency (SCID).These include CD132 expression for T-B+NK- profile ,lack of CD132 expression on T cells indicate X linked SCID whereas its presence indicates a JAK 3 defect to be further confirmed my molecular studies. Study of CD127 expression by flow cytometry in T-B+NK+ will help identify IL7alpha receptor(IL7Rα) defect.In the T-B-Nk+ possibility of RAG1/RAG2 and artemis gene defects can be assessed. In cases with T-B-NK- the uric acid levels help differentiate ADA (normal uric acid levels)and PNP(reduced uric acid levels) defects . ,In less severe forms of CID the isolated CD4 deficiency with lack of HLADR expression indicates MHC classII defect and isolated reduced CD8 counts with ZAP70 deficiency will indicate MHC class I defect. Patient with markedly reduced IgG ,IgA, IgE levels and increased or normal IgM levels CD40 ligand or CD154 expression b flowcytometry can help pick up X-linkedHper IgM which in some may require sequenceing for CD40 ligand gene. The increase in dual negative (CD4-/CD8-/CD3+) TCRαβ positive T cell subsets in the correct clinical scenario can help diagnose Autoimmune lymphoproliferative syndrome(ALPs) which can then be further confirmed by germline OR somatic mutations in FAS The primarily antibody deficiency with absolute lack of B cells in basic lymphocyte subset panel will indicate X linked BTK deficiency in 85% of cases. The flowcytometry to look for BTK expression on monocytes is available in specialized centres this can be further confirmed by looking for specific mutation of. In CVID cases and adults with reduced immunoglobulin levels the B cell subset with reduced switched Memory B cells and plasmablast can be identified using exetended B cell sebset analysis. Though many of these tests are feasible in referral PID centres in multispeciality settings IEI can easily pick up by basic lymphocyte subset analysis along with immunoglobulin levels and clinical evaluation for atleast 3 to 4 warning signs. The further evaluation can then be done by either by molecular studies like next generation sequencing or whole genome clinical sequencing or referral to specialized cetres. The variables of lymphocyte counts including physiological, circardian, therapy and infection related should be borne in mind while analysing the lymphocyte subests.

Dr Manisha Madkaikar
National Institute of Immunohematology, Mumbai

The role of flow cytometry in the array of diagnostic tools for diagnosis of IEI-an overview

Human inborn errors of immunity (IEIS) are a group of heterogeneous disorders with underlying monogenic defects affecting the immune system. The clinical spectrum varies from increased susceptibility to infections, autoimmunity, auto-inflammatory diseases, allergy, bone marrow failure, and/or malignancy. The updated classification of International Union of Immunological societies (IUIS-2022) have enlisted a total of 485 inborn errors of immunity.

The most important challenge in the diagnosis of IEI, is the phenotypic and genotypic heterogeneity. This heterogeneity makes genetic diagnosis difficult and urges extensive, rapid, and yet easy-to perform tests for proper evaluation and categorization of patients. Flow cytometry (FCM) has gained great importance in the diagnosis of these disorders as it enables measurement of multiple characteristics of single cells in a peripheral blood. The immunophenotypic and functional evaluation of IEIs is also useful for monitoring and predicting clinical outcomes, and to determine the immunological significance of newly identified genetic defects.

Various flowcytometry based assays including lymphocyte subset analysis, expression of various proteins defective in patients with IEI and different functional assays are available for evaluation of immune system in patients with IEI. The lecture will focus on the various tools available and their applications in the diagnosis of IEIs.

Dr Manas Kalra
Sir Gangaram Hospital
New Delhi

What happens after flow? Role of genomics & phenocopies

Among the early described immunodeficiencies, X-linked agammaglobulinemia, severe combined immunodeficiency and Wiskott Aldrich syndrome constituted the majority. With advances in genetics and diagnostic methodology, the spectrum of patients with symptoms of immunodeficiency broadened tremendously. Increased susceptibility to infection, symptoms of autoimmunity, atopy, autoinflammation and predisposition to malignancy form the new spectrum of immunodeficiency. In addition, recent years have brought reports of gain-of-function mutations causing these symptoms, whereas, in the past, the predominant focus was on mutations causing absence or decreased function of the encoded protein. Overall, the number of genetically defined forms of primary immunodeficiency has demonstrated rapid growth in recent years

This reflects increased recognition of the varied phenotypes associated with immune dysfunction, as well as increased availability of whole exome sequencing (WES) and, in some cases, whole genome sequencing (WGS). In this era, treatment decisions can be informed by the results of genetic testing

"Phenocopies of PIDs" is a special group of immune deficiencies that are caused by a somatic mutation (rather than genomic mutation) or autoantibodies against various cytokines thereby producing clinical manifestations similar to other immunodeficiencies. These diseases do not follow a Mendelian pattern of inheritance. Phenocopies of PIDs have been classified separately by the International Union of Immunological Societies (IUIS).

Dr Ananthvikas Jayaram
Neuberg Anand Reference Laboratory
Bangalore

Lymphocyte related inborn errors of immunity – a primer to lymphocyte subsets and their roles

Lymphocytes are one of the main players in the adaptive immune response and are present in large numbers in circulation and in lymphoid organs. These cells respond to foreign antigens after the activation of the innate immune system, often migrating to the sites of infection to directly destroy microbes, or to further activate B cells to respond to microbial antigens. The major lymphocyte subsets in circulation are T cells, B cells and Natural Killer cells.

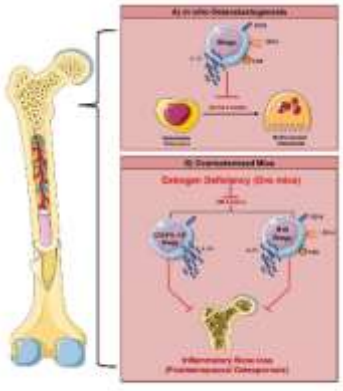
Abstracts - Oral Papers Basic

B1 - Functionally compromised B-regulatory cells (Bregs) enhance inflammatory bone loss under estrogen deficient osteoporotic conditions

Leena Sapra & Rupesh K. Srivastava
AIIMS, NEW DELHI

Background: In 2002, a subset of B cells has been identified named as regulatory-B cells-Bregs. Several evidence and our group suggest that Bregs plays a vital role in inflammatory disease conditions including bone remodeling. Nevertheless, no study till-date has revealed the functional activity of Bregs in osteoporotic-condition. To our knowledge, this study for the first time investigated the anti-osteoclastogenic potential of Bregs osteoporotic conditions.
Material and Methods: Firstly, to investigate the bone-heath modulating potential of Bregs co-culture between bone-marrow-cells-BMCs and Bregs was carried-out. TRAP-staining was performed for evaluating presence of multinucleated-TRAP-positive-osteoclasts. Further transwell and IL-10 neutralization assay was performed. For in-vivo experiments C57BL/6 mice were divided to two groups: sham and ovariectomy (Ovx-ovaries removed bilaterally) and after 45 days various osteoimmune parameters were analyzed. Lastly, we corroborated our findings in human subjects.

Results: Our in-vitro co-culture assays suggested that Bregs suppressed the RANKL mediated differentiation and functional activity of osteoclasts in a cell-ratio dependent-manner. Further, trans-well and neutralizing-antibody experiments revealed that Bregs inhibited osteoclastogenesis in an IL-10-cytokine dependent-manner. Interestingly, our in-vivo data suggest that frequencies of both total B cells (CD19+IL-10+) and B10 Bregs (CD19+CD1dhiCD5+IL-10+) were significantly-reduced in bone marrow and spleen of Ovx mice. In addition, ELISA data indicated towards significant-reduction in the IL-10 cytokine levels of Ovx-mice. Of note, CFSE-suppression assay indicated that Bregs harvested and generated from Ovx-mice showed-reduced tendency to suppress the proliferation of CFSE-labelled effector T-cells in comparison to sham-group thus suggesting towards compromised-immunosuppressive potential of Bregs in PMO. Moreover, anti-osteoclastogenic potential of Bregs compromised in Ovx-mice. In consistent to this, our in-vitro data further demonstrated that exogenous addition of 17β-estradiol significantly-enhanced the percentage of IL-10 producing-Bregs and its efficacy to modulate the immune-cells. Lastly, we observed that percentage of Bregs (CD19+CD38hiCD24hiIL-10+) was found to be significantly-reduced in the PBMCs of PMO patients.



Conclusion: Thus, altogether our-research demonstrates Bregs direct involvement in inhibiting osteoclastogenesis. Furthermore, our pre-clinical and clinical-data revealed that numerical defect in the percentage of Bregs along-with its reduced potential to produce IL-10 cytokine and compromised-immunosuppressive-potential further enhance bone-loss in Ovx-mice and in the osteoporotic-patients. Altogether, our study for the first-time explores the "Immunoporotic" role of Bregs in bone-health.

B2 - Modulation of Host Macrophage Polarization by Leishmania donovani Exosomes: A Polyamine Metabolism Perspective

Radheshyam Maurya & Prince Sebastian

University of Hyderabad

Leishmaniasis is a vector-borne, intracellular protozoan parasitic disease. There are three clinical forms of the disease Cutaneous Leishmaniasis (CL), Mucocutaneous Leishmaniasis (MCL), and Visceral Leishmaniasis (VL).VL is caused mainly by Leishmania donvani and L. infantum and is fatal if left untreated. Exosomes are small (~30-100nm) Extracellular Vesicles of endocytic origin and play a major role in the host-pathogen interaction and intracellular communication. The Leishmania exosomes are carriers of many virulence factors to the host cell for better survival of the pathogen inside the harsh niche. The exosome-mediated immune metabolic regulation of host macrophage polarization needs to be better studied. The present study interrogates the role of Leishmania-specific exosomes in the immune modulation of the host macrophage polarization from the M1 (Classically activated) to M2 (Alternatively activated) phenotype by activating the polyamine metabolism. The Leishmania exosomes are isolated (Ultracentrifugation) and characterized with Transmission Electron Microscopy, Dynamic Light Scattering, and Western blot with Anti-GP63 antibody (Leishmania exosome marker). Chromatographic (TLC, HPLC, LC-MS) and Semi-Quantitative Flow cytometry analysis estimate exosome PAs and other metabolites. Interestingly, our result shows that the Leishmania exosomes were enriched with polyamines. Furthermore, Flow cytometry analysis and Confocal microscopy were carried out for the macrophage uptake study of the Leishmania exosomes. The macrophage polarization study with Western Blot (WB), Flow cytometry, and RT-PCR analysis shows the macrophages up-taken exosomes and polarized the macrophages by increasing the Arginase I expression and activity. The result also indicates that exosome stimulation enhances the phagocytic index and infectivity by creating a presumptive environment. The L. donovani exosomes-mediated polarization of host macrophages from M1 to M2 macrophage phenotype. The macrophage polarisation drives the polyamine pathway instead of the iNOS pathway, which helps the parasite survive inside the host macrophages.

The Cytometry Society-India
15th Annual Conference & Workshops
26th -29th October, 2023
Theme: From Basics to Multi-Omics

B3 - The interplay of sMAdCAM and sVCAM in cellular immune dysfunction responses accompanying HIV-1 infection

Sapna Yadav, Nandini Kasarpalkar, Shilpa Bhowmick, Snehal Kaginkar, Pranay Gurav, Satyajit Musale, Sameen Khan, Denna Shebin, Sachee Agrawal, Geeta Natraj,Jayanti Shashtri, Vikrant Bhor, Vainav Patel
ICMR- National Institute for Research in Reproductive and Child Health

Introduction: HIV infection is associated with abnormalities in the synthesis and expression of various adhesion molecules. Vascular Cell adhesion molecule (VCAM) mediates the adhesion of immune cells to activated vascular endothelium and their subsequent migration whereas Mucosal Addressin Cell adhesion molecule (MAdCAM) is associated with migration of immune cells to the gut. There are reports which suggest that people living with HIV have a higher risk of Cardio vascular diseases. Also, Gut homing property of integrin α4β7 contributes toward establishing HIV infection in the gut. Hence it becomes important to understand the association between MAdCAM/VCAM with various disease progression markers and immune responses in HIV infected individuals.

Objective: To evaluate correlation between cell adhesion molecules and associated immune response in HIV infected individuals with or without antiretroviral therapy (ART).

Method: sMAdCAM and sVCAM levels were estimated in stored plasma samples using ELISA in (n=23) ART naïve and (n=46) on ART HIV infected individuals. Evaluation of T cell activation on the basis of HLADR and CD38 and exhaustion on the basis of PD-1 expression and frequency of B cell (CD-19+) was carried out by multiparametric flow cytometry using stain/lyse/wash technique and atleast 1,00,000 lymphocytes were acquired. Aquired samples were analysed using FlowJo and Graphpad Prism.

Result: sVCAM level was found to be significantly lower in the HIV infected group receiving ART compared to ART naïve individuals. sMAdCAM level was observed to be associated positively with absolute count of CD8+ T cells as well as B cells among ART naïve individuals, however among ART receiving individuals the association was observed only with absolute count of B cells.

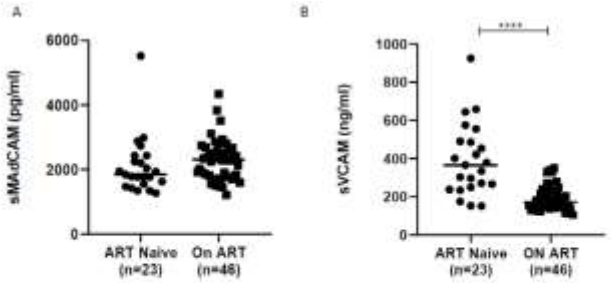


Figure 1: Plasma sMAdCAM and sVCAM levels among HIV infected individuals. Distribution of sMAdCAM-1 (soluble Mucosal addressin cell adhesion molecule) levels (A) and sVCAM (soluble vascular cell adhesion molecule) in plasma of HIV infected cohort of antiretrovial therapy (ART) naive (circle) and ART receiving (square) cohort. Median is depicted with a line. Statistical analysis was done by Mann-Whitney test using Graphpad Prism 8.

Conclusion: Association of MAdCAM and VCAM with cell mediated and humoral responses against HIV needs to be evaluated further in the context of other systemic immune dysfuntion such as T cell activation and exhaustion.

B4 - Super Nova Tandem Dyes: Polymer Dyes with superior stain index and minimal non-specific staining

Jasmine Gouda1, Vishal Gehlot1, Anandh Banu1, Milind Shreykar1, Lucille Lassalle2, Maxime Moulard2 and Shiva Ranjini1.
1 Beckman Coulter India Private Limited, Bangalore Development Centre, Bangalore, INDIA.
2 Biomarkers, BioCytex, Marseille, FRANCE

With the advent of Multicolor Flow Cytometry, researchers and clinicians have been able to explore complex specimen populations by introducing additional fluorochromes into the panel. SuperNova SN v605 and SN v786 facilitate further expansion of the color palette.

Derived from the core SN v428 dye, these conjugates have maximum excitation at 414 nm and their expression is optimally detected using the flow cytometer's 610/20 and 780/60 nm bandpass filters or equivalent. By enabling analysis of dimly expressed and low abundance markers without increasing background, they help to clearly discriminate between positive and negative cells.

This poster shows development and performance comparisons of SuperNova conjugates and similar commercially available conjugates in terms of stain index and background.

Highlighting Features:

• **High stain index**: SN v605 and SN v786 conjugates demonstrate high stain index for their respective channels, and show significantly reduced non-specific staining, thereby providing greater confidence in results.

• **Minimal non-specific staining due to proprietary in situ stabilization method:** Beckman Coulter has developed an in-situ formulation involving certain additives to stabilize these dyes, thereby controlling non-specific staining without hampering conjugate performance.

• **State-of-the-art manufacturing process**: SNv605 and SNv786 dyes result from the attachment of an appropriate acceptor dye to a polymer backbone, creating tandem dyes with similar absorbance but different emission properties. The manufacturing processes of Analyte Specific Reagent conjugates are optimized and standardized to maximize FRET, ensuring greater stability and consistency across lots, and to meet cGMP requirements.

Conclusion:

The manufacturing process of SN v605 and SN v786 enables the production of conjugates with unique photo-physical properties, thus providing significantly brighter conjugates for flow cytometry applications. A proprietary formulation leveraging unique additives minimizes non-specific staining for greater confidence in flow cytometry staining results and an improved staining index.

Abstracts – Poster Papers Basic

BP1 Advancing solid tumor immunotherapy via in vivo T-cell engineering using lentiviral implants

Hiren Dandia, Deepak Sharma, Arvind Ingle, Shubhada Chiplunkar, Prakriti Tayalia

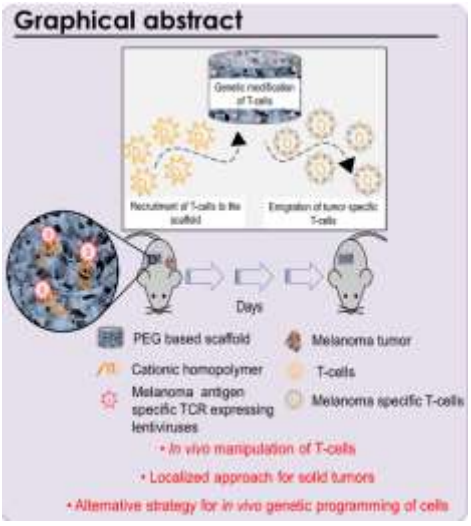
Indian Institute of Technology Bombay

Background: Genetic engineering of T-cells with tumor antigen-specific CAR or TCR in laboratories is a laborious, time-consuming and expensive process. Additionally, engineered T-cells often lose their effectiveness due to challenges posed by transition from controlled in vitro culture conditions to complex and dynamic in vivo environment.

Aim: Our study aims to develop a strategy using a biocompatible implant for in vivo genetic engineering of T-cells with tumor antigen specific TCRs, while also evaluating its efficacy in treating pre-clinical solid tumor models.

Method: The implant was created using polyethylene glycol (PEG)-based polymerization technology and surface modified by attachment of a cationic homopolymer, thereby, enhancing its ability to bind lentiviruses. These lentiviruses expressed tumor antigen-specific TCRs, enabling genetic programming of T-cells to specifically target tumors. The implant was tested to assess its biological properties for suitability within the body. In a melanoma tumor model developed in mice, efficient transduction of T-cells was studied and characterized through tumor reduction and comprehensive cytokine analysis.

Results: The developed implant could immobilize lentiviruses and also recruit cells. Upon subcutaneous implantation in mice, it exhibited lack of systemic inflammation while actively attracting T-cells to the site. Intriguingly, bioluminescence data revealed that gene delivery mediated by this implant surpassed conventional methods involving bolus injection. When implanted in mice with tumors these implants recruited and genetically programmed host T-cells with tumor antigen specific TCRs. As a result, there was significant reduction in tumor size and a widespread release of anti-tumor cytokines throughout the body. Immunophenotyping indicated the presence of T-cells modified genetically in vivo, which were found in tumor as well as in secondary lymphoid organs.



Conclusion: This strategy tackles the obstacles encountered in in vitro genetic programming of T-cells, showcasing an alternative strategy, namely, implant-mediated in vivo gene delivery, which can be widely applicable for solid tumor immunotherapy.

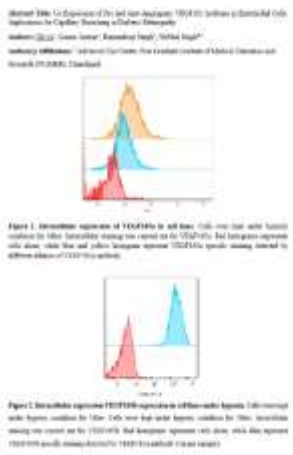
The Cytometry Society-India
15th Annual Conference & Workshops
26th -29th October, 2023
Theme: From Basics to Multi-Omics

BP2 - Co-Expression of Pro and Anti-Angiogenic VEGF165 Isoforms in Endothelial Cells: Implications for Capillary Branching in Diabetic Retinopathy.

Divya, Gaurav kumar, Ramandeep Singh, Nirbhai Singh

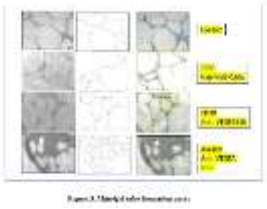
Postgraduate institute of medical education and research, Chandigarh

Purpose: VEGF165 (Vascular Endothelial Growth Factor 165) serves as a linchpin in ocular angiogenesis. This study scrutinized the interplay between VEGF165a (pro-angiogenic) and VEGF165b (anti-angiogenic) isoforms using an in vitro endothelial cell model, explored the co-expression of these isoforms, and assessed the impact of inhibiting VEGF165 isoforms on retinal capillary branching under hypoxic conditions simulating those seen in diabetic retinopathy.



Methods: To mimic diabetic retinopathy's hypoxic milieu, human umbilical vein endothelial cells (HUVECs) underwent normoxic (20% O2) and hypoxic (1% O2) exposure. Custom antibodies targeting VEGF165a and VEGF165b enabled flow cytometric assessment of intracellular expression. The effect of neutralizing the pro-angiogenic and anti-angiogenic isoforms of VEGF on retinal capillary branching was studied by using isoform-specific custom antibodies, followed by a Matrigel tube formation assay conducted under hypoxic conditions.

Results: Hypoxia led to an upregulation of VEGF165a and VEGF165b gene and protein expression in HUVECs. Flow cytometry confirmed intracellular co-expression of both isoforms. Notably, VEGF165a neutralization significantly reduced capillary branching intervals, surpassing the impact of VEGF165b neutralization. The use of Avastin completely blocked branching, validating our approach.



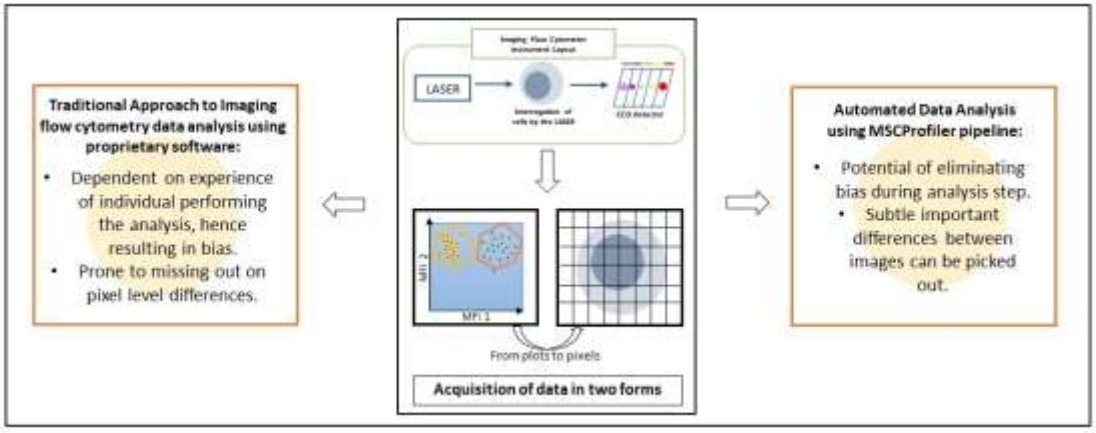
Conclusion: This study reveals the pivotal roles of VEGF165 isoforms in regulating capillary sprouting, a fine control on angiogenesis switch under hypoxic conditions similar to those observed in diabetic retinopathy. Targeting the pro-angiogenic VEGF165a, leaving VEGF165b intact, presents a refined potent strategy to impede angiogenic signalling. These findings deepen our understanding of angiogenic control and propose novel therapeutic avenues for angiogenesis-linked diseases.

BP3 - MSC Profiler- a novel pipeline towards promoting unbiased imaging flow cytometry data analysis.

Ayona Gupta, Risani Mukhopadhyay, Lakshmi Balasubramanian, Uttara Chakraborty

Manipal Institute of Regenerative Medicine

The combination of imaging and high-throughput acquisition of flow cytometry has culminated to give us the imaging flow cytometry platform. Each dot in the plot is accompanied by its image information, therefore there is generation of an enormous amount of data. Thus, as flow cytometry enters the realm of 'big data' there is an increasingly growing effort to apply machine learning or deep learning concepts in cytometric data analysis. To capitalize on the full potential of the IFC data, we require image analysis software/ tools that can give us unique identifiers of cellular structure that otherwise might be overlooked. Here, we demonstrate a pipeline named 'MSCProfiler' which has been made using the CellProfiler software, with the goal of identifying heterogeneity with the same passage of Mesenchymal Stem Cells (MSCs) isolated from human exfoliated deciduous tooth (SHEDs). Over 50,000+ single-cell images of stem



cells from exfoliated human deciduous teeth (SHEDs) were run through the MSCProfiler to obtain information on texture and granularity features, and fluorescence intensity. Cells were stained for CD73 PerCP-Cy5.5, CD44 V450, and CD45 PE along with Sytox Green which was used as a live dead discriminator in this panel designed for a single camera CCD detector (6 channels) of Amnis ImageStream Mk II. Additionally, brightfield information of these cells was also acquired. The features obtained from our pipeline were further used to distinguish cells into three distinct categories of cells based on their sizes. We have also validated the pipeline using Wharton Jelly (WJ) MSCs to extract similar features of the cells. Further, CellProfiler Analyst can be used to build a classifier that can distinguish between the two sources of MSCs based on the image data extracted from the MSCProfiler.

BP4 -Immunological Profiling Reveals Unique Features of Melioidosis Infection in the Indian Population: Insights from Immune Phenotyping and Activation Markers

Ranita Ghosh Dastidar[1,2]*Sparsha Pallen[1,2], Sindhura Lakshmi Koulmane Laxminarayana[3,] Piyush Behari Lal[1,4], Somasish Ghosh Dastidar[1,5], Chiranjay Mukhopadhyay[1,4#],

[1]Center for Emerging and Tropical Diseases, Kasturba Medical College, Manipal, India; [2]Department of Biochemistry, Kasturba Medical College, Manipal, India; [3]Department of Pathology, Kasturba Medical College, Manipal, India; [4]Department of Microbiology, Kasturba Medical College, Manipal, India; [5]Centre for Molecular Neurosciences, Kasturba Medical College, Manipal, India

Melioidosis, a life-threatening bacterial disease caused by Burkholderia pseudomallei, remains poorly understood due to its status as a neglected tropical-disease and immuno-profiling has been least explored globally. Immunophenotyping offers insights into immune-cell types, quantities, activation levels, and functionalities. The objective of this study is to assess the immune-subsets and their activation-status during active B. pseudomallei infection to study their immune marker expression-pattern. The immune-cell activation markers, HLA-DR and CD38, and immune-cell metabolic status markers, GLUT1 and CD98, can enhance understanding of immune-cell functions during response to infections. 6 individuals were included in this study to perform a thorough immune phenotyping to understand the immunological features distinguishing Melioidosis infection in Indian population. Peripheral blood samples were collected from melioidosis patients and healthy controls with no active infections, with/without non-infectious complications under control. Flow cytometry was performed to quantify the TBNK immune-cell subsets and monocytes. Differences were noticed in specific sup-populations of circulating lymphocytes relative to matched control groups. The CD4+:CD8+T-cell ratio remained within the normal range, 1.8±0.6 in the infected individuals indicating no immuno-compromised phenotype at early stage of infection. However, an impaired humoral response was evident as seen by reduced expression of T-follicular helper cells (CD4+CXCR5+), accounting for only 0.51±0.1% of lymphocytes compared to 3.85±0.6% in the control group. A decrease in the activation of certain immune-cell subsets is observed, particularly CD8+HLA-DR+T-cells (0.2±0.1%) when compared to controls (1.88±0.3%). There is a difference noted in the expression of CCR7, associated with memory function, in CD8+T-cells. This ongoing study highlights the significance of the alterations in immune-profiles in melioidosis patients. Notably, T-cell counts and activation-markers differ across the study groups, emphasizing the need to understand these distinctions and their potential impact on therapeutic approach as well as for the development of promising immunological biomarkers for Melioidosis diagnosis.

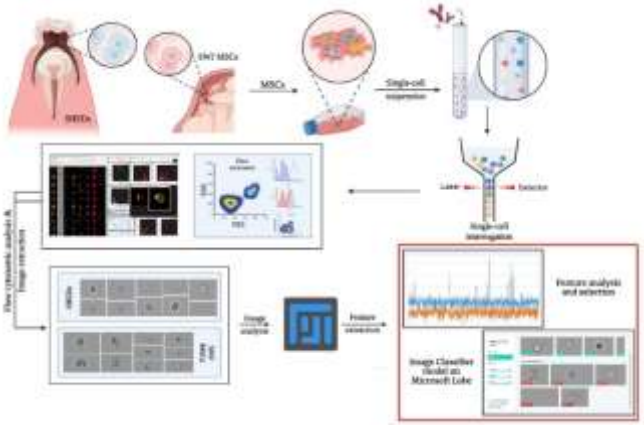
Keywords: Immunophenotyping, Melioidosis, *Burkholderia pseudomallei.*

BP5 - Towards understanding the mesenchymal stem cell morphometric heterogeneity using single-cell image analyses algorithms

Risani Mukhopadhyay. Dr. Uttara Chakraborty, Dr. Keerthana Prasad

Manipal Institute of Regenerative Medicine, Bengaluru, Manipal Academy of Higher Education, Manipal, India

Mesenchymal stem cells (MSCs) are one of the most widely studied multipotent stem cells that have been recognized in biotherapeutics. This is because, these cells play multiple vital roles in tissue repair and regeneration in vivo. However, as they are derived from a variety of sources with different tissue origins, MSCs constitute a heterogeneous population of cells with ambiguous in vitro behavior. Even if the cells are clonally derived, each cell may differ intra-clonally from one another in their functional and morphological properties. This cellular heterogeneity brings along diversity in therapeutic properties making it necessary for standalone quality check (QC) procedures to perform the first round of quality assurance for the MSCs before their translation. In this project, we have studied the immuno-heterogeneity of two sources of human MSCs using the technology of imaging flow cytometry (IFC) in a multiparametric approach. The scope of the study lies in the identification of subpopulations that display altered expression patterns of surface markers, and the quantitative analysis of cellular structures of those subpopulations, based on their brightfield single-cell images acquired using IFC. This has been further analyzed using ImageJ to quantify them and extract features of the cells by specifically describing their morphometric characteristics based on shape and texture. Alongside we have utilized Microsoft Lobe to create an Image classifier to identify the scope of creating a model that can classify between the two cell types and multiple subpopulations that arise due to the identified heterogeneity. Lastly the identification of descriptors that efficiently recognize these heterogeneous populations could further be utilized for the creation of a label-free score chart or deep learning model that can be used to screen MSCs isolated from different sources across passages, for optimal usage in therapy.

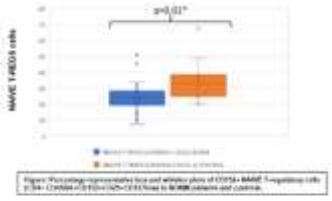


The Cytometry Society-India
15th Annual Conference & Workshops
26th -29th October, 2023
Theme: From Basics to Multi-Omics

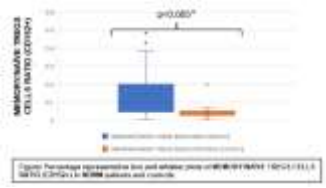
BP6 -Analysis of T-regulatory cells in Multiple Myeloma.

MOHINI VIG, SANDEEP RAI, SHWETA DUBEY AND RITU GUPTA
AMITY UNIVERSITY

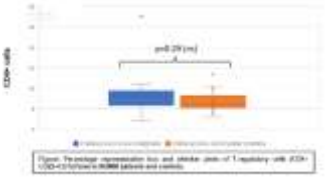
Introduction: Multiple myeloma (MM) is a haematological malignancy characterized by expansion of plasma cells in bone marrow. Active disease is characterized by perturbations in immune system which affect bone marrow microenvironment. Regulatory T cells (Tregs) represent an important subset of CD4 T cells which have regulatory and immunosuppressive functions. Data from various haematological malignancies have shown impact of elevated Tregs in the disease process. This study was designed to study the role of Tregs in disease pathogenesis of MM.

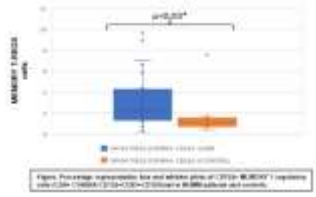


Aim/Objective: This study was aimed at assessing the frequency of Tregs in peripheral blood of newly diagnosed multiple myeloma (NDMM) patients and controls.



Method-Multi-colour flow cytometry was used to analyse the status of T-regulatory cells in peripheral blood of NDMM patients (n=31) and controls (n=11). Tregs were defined as CD4+CD25+CD127low. Tregs were further defined as naïve and memory subsets on the basis of CD45RA expression. Naïve Tregs were defined as CD4+CD45RA+CD25+Cd127low and memory Tregs as CD4+CD45RA-CD25+Cd127low. Naïve and memory Tregs were also analysed for presence of CD152/CTLA-4 expression. Difference in Treg frequency between patients and controls was compared by applying Mann-Whitney U-test.





Results and Discussion: Frequency of Tregs was elevated in NDMM as compared to controls, however it was not statistically significant. (Median percentage in patients=8.148 vs 5.914 % in control, respectively). The percentage of naïve Tregs expressing CTLA-4 was significantly low in NDMM (median=24.18) as compared to control (median=33.01; p=0.01). Similarly, memory Tregs expressing CTLA-4 (p=0.03) was also significantly high in patients (median= 2.18 vs 1.13 in controls). Furthermore, the ratio of memory vs naïve Tregs expressing CTLA-4 (p=0.005) was also significantly high in patients (median=0.101) as compared to controls (median=0.038).

Conclusion-The up-regulation of these cells in multiple myeloma may indicate their involvement in altering the tumor microenvironment and promoting malignant B-cell survival in MM.

The Cytometry Society-India
15th Annual Conference & Workshops
26th -29th October, 2023
Theme: From Basics to Multi-Omics

BP7 - Studying the dynamics of recall T-cell response post third booster dose of COVISHIELD/COVAXIN among healthy adult population.

Tejaswini Pandey, Amit Kumar Singh, Harsha Palav, Varsha Kalsurkar, Nandan Mohite, Pranay Gurav, Vaibhav Daund, Gaurav Paradkar, Vikrant Bhor, Vainav Patel

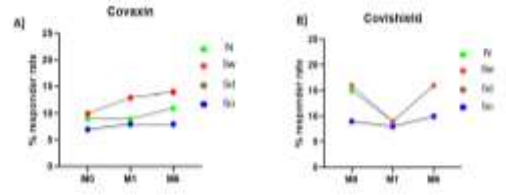
ICMR- National Institute for Research in Reproductive and Child Health

Introduction: Post third wave of COVID-19 primarily driven by Omicron Variant of Concern, India's COVID-19 vaccination programme had proposed to initiate a precautionary third dose to health workers, frontline workers & individuals above 60 with co-morbidities.

Study Objective: To characterize SARS-CoV-2 specific cellular immune responses after homologous precautionary third dose of COVISHIELD/COVAXIN at different time points.

Methods: Participants (n=33) were recruited from ICMR-NIRRCH, Mumbai, prior to taking booster dose. Whole blood samples were collected during 4 different time points. 20ml (EDTA vacutainer) was used to isolate and cryopreserve peripheral blood mononuclear cells (PBMC's). Recall T-cell response was evaluated by Intracellular Cytokine Staining Assay & Flow cytometry on cryopreserved PBMCs using four SARS-CoV2 specific antigenic peptides: N, Sw (Wild type spike protein), Sd (Delta spike protein), So (Omicron spike protein). We assessed degranulation (CD107a), cytokines (IFN-γ, IL-2, TNF-α), chemokine (MIP-1-β) within different T cell subsets.

Results: Responder rate: For covaxin, responder rate observably increased for N & Sw. However, the same for covishield decreased from M0 to M1 for Sw and increased from M1 to M6. For covaxin: cross-sectional analysis revealed trend in boosting for N from M1 to M6 and with respected to Sw, we observed boosting from M0 to M1, whereas it decreased from M1 to M6. Similar boosting patterns were observed in longitudinal analyses for both N and Sw. For covishield: cross sectional analysis revealed trend in boosting for Sw from M1 to M6 and longitudinal analysis showed boosting from M0 to M1 and further till M6. For covishield, in both the analyses we observed response against N thus indicating presence of hybrid immunity. Response against Sd and So among both covaxin & covishield receiving participants showed evidence of cross reactivity.



% Responder rate: Studying the dynamics of cell mediated immunity in individuals who have received different COVID-19 precautionary third dose vaccine (A) Covaxin (B) Covishield, at respective time intervals i.e. before booster (M0), 4 weeks after booster (M1) and 6 months after booster (M6).

Conclusion: Both vaccines showed trends for boosting over the follow up period, with evidence for cross-reactive immune responses and hybrid immunity.

BP8 - A comparative evaluation of immuno-dominant and cumulative HCMV specific cellular responses in the context of pregnancy

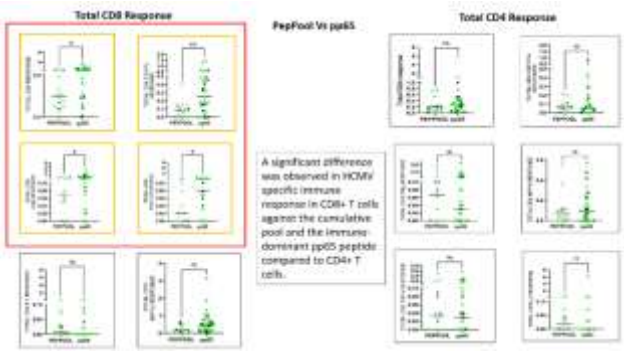
Pranay Gurav, Harsha Palav, Shilpa Velhal, , Varsha Padwal, Sayantani Ghosh, Gauri Bhonde, Purnima Satoskar, Vikrant Bhor, Vainav Patel

ICMR- National Institute for Research in Reproductive and Child Health

Introduction: Human Cytomegalovirus (HCMV) is an opportunistic pathogen causing severe threat in immune-compromised individuals. Pregnancy is a state of altered-immune system, making women more susceptible to HCMV infection or reactivation which further exposes new-borns to high risk of viral pathology. pp65 (ppUL83) is major tegument protein of HCMV and an important target of humoral as well as cellular HCMV specific immune response. The CMV Peptide pool contains 42 peptides representing pp50, pp65, IE1, IE2 and gB proteins which are important at various stages of viral life cycle. Aim- Comparative evaluation of HCMV specific immune response in pregnant women against cumulative pool (PepPool) and an immuno-dominant peptide 65kDa phosphoprotein (pp65) of HCMV (strain AD169).

Methods: HCMV specific immune response against pp65 (1µg/ml)- JPT™ Peptides Technology and PepPool (2µg/ml)-Mabtech™ were evaluated using cryopreserved PBMCs collected from pregnant women(n=16) by performing intracellular cytokine staining and multi-parametric flow cytometry. HCMV specific T cell responses were analyzed using fluorescently conjugated surface antibodies i.e. CD3, CD4 and CD8 and memory subsets for robust functional analysis were identified based on CD45RA and CCR7. The functional markers included were CD107a (degranulation), (cytokines) IFN-γ, IL-2, TNFα and (chemokine) MIP1β. The samples were acquired on BD FACSymphony™ A3 and data was analyzed using FlowJo™. The statistical analysis was performed using GraphPad Prism™.

Results: A significant difference was observed in HCMV specific immune response in CD8+ T cells against the cumulative pool and the immuno-dominant pp65 peptide compared to CD4+ T cells. Also the percent expression of degranulation marker (CD107a), Th1 cytokine production (TNFα and IFNγ) and chemokine (MIP-1β) was significantly higher in CD8+ T cells stimulated by pp65 compared to PepPool.



Conclusion: The study highlights the importance of immuno-dominant pp65 peptide for immune monitoring studies compared to a cumulative pool of peptides representing a set of HCMV derived antigens.

Abstracts - Oral Papers Clinical

C1 - Proposal of Mantle cell lymphoma Immunophenotypic scoring system (MCL-IPT), boon to early and reliable diagnosis.

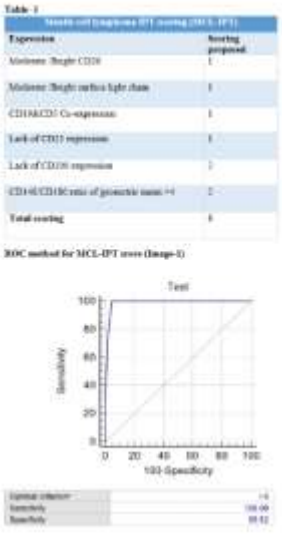
Dr. Pradeep Arumugam[1] Dr. Avinash Gupta[1] Dr. Neha Singh[1], Dr. Pratheek Das[1], Dr. Rajul Bodhka[1], Dr. Akshaya Mandloi[1], Rohitkumar kori[1], Nilesh Dhole[1], Dharmendra Mishra[1], Dr. Sujit Kumar [2], Dr. Anil Singh [2], Dr. Malini Garg[2].

Department of Hematoncopathology[1] and Department of adult hematolymphoid unit [2] of Tata memorial Centre (HBCH and MPMMCC) Varanasi.

Introduction: Mantle cell lymphoma (MCL) is a CD5 positive mature B cell neoplasm (B-NHL) with an aggressive clinical course. At times due to varied clinical presentation and unusual morphology diagnosis becomes very challenging and requires other time-consuming ancillary testing. Multi-parametric flowcytometry (MFC) plays a pivotal role in providing authentic and rapid diagnosis of mature B cell neoplasms. We hereby proposing a (MCL-IPT) based on MFC.

Materials and methods: In this retrospective study from 2022 to 2023, we assessed the results of MFC analysis in 74 patients of B-NHL. Diagnosis of all the non CLL cases was confirmed by histopathology/molecular studies. Total of 74 (45 Peripheral blood samples and 29 bone marrow aspirate) samples were processed by lyse-stain-wash method using a 10-13 color two tube panel. Acquisition was done on Beckman Coulter Dxflex 13 color, 3 lasers flowcytometer. Data analyzed by Kaluza software Version 2.1. Based on the CD19/CD5/CD20/CD23/CD148/CD180/CD200 specific expression patterns we postulated a diagnostic MCL-IPT (Table 1).

Results: Our study results included 74 patients with mean age of 59.48, Males:53; Female:21. The distribution were CLL-49(66.21%), MCL -7(9.45%), follicular-6(8.10%), DLBL-2(2.70%), LPL-2(2.70%), Burkitts-2(2.70%), splenic B-cell lymphoma/leukemia with prominent nucleoli (SBLPN)-2(2.70%), SMZL-2(2.70%) and one HCL (1.35%). MCL IPT scoring done and cutoff of more than score >5 was derived by using ROC method with a sensitivity of 100%, specificity of 95.2% and p value <0.001(Image-1).



Conclusion: Multi-parametric Flow Cytometry (MFC) emerges as an indispensable and reliable diagnostic tool for the early detection of Haematolymphoid Neoplasms. Introduction of this novel MCL-IPT scoring system will help in an accurate and rapid diagnosis of Mantle Cell Lymphoma (MCL), especially in rare and complex cases where distinguishing MCL from other rare B cell Non-Hodgkin Lymphomas (NHL) poses a significant challenge. However, comprehensive extensive studies with larger sample sizes are further needed for validating the diagnostic potential of this score.

C2 - Role of normalized flow cytometry assessment of CD5 expression in B cell chronic lymphoproliferative neoplasms

Sindhura Lakshmi Koulmane Laxminarayana, Soundarya Ramanathan

Kasturba Medical College, Manipal, Manipal Academy of Higher Education

Introduction: Analysis of CD5 expression by in chronic lymphoproliferative disorders (CLPD) is essential. It is invariably expressed in chronic lymphocytic leukemia/small cell lymphoma (CLL) and mantle cell lymphoma (MCL) with rare exceptions. It may be expressed in small levels in other B cell neoplasms as well. The assessment is generally qualitative and subjective. In this study, we aimed for normalized and objective analysis of CD5 expression in B CLPD to differentiate between various subtypes.

Material and methods: A retrospective observational study was performed on patients who underwent flow cytometry analysis on peripheral blood or bone marrow aspirate for B CLPD. Eight color flow cytometry files were analysed for CD5 expression in B cells. CD45+CD19-CD5+ T cells were considered as positive internal control. CD45+CD19-CD56+ NK cells were considered as internal negative control. Data normalization was performed by transforming the MFI values of the test population to a common scale using the following equation: Final relative fluorescence intensity = MFI of the test sample/MFI of the internal control. Statistical analysis was performed to study the significance of difference of CD5 expression in different B CLPD. A p value of <0.05 was considered significant.

Results: A total of 116 patients were included in the study. There was higher and homogeneous CD5 expression in CLL and MCL. Marginal Zone Lymphoma (MZL) and Hairy Cell Leukemia (HCL) had CD5 expression comparable with the normal internal control. When CD5 expression is partial or weak or heterogeneous, weak expression of CD20 and surface light chain are most predictive of CLL.

Conclusion: Using normalized CD5 expression helps in objective assessment of B CLPD. Variable CD5 expression may be seen in diffuse large B-cell lymphoma, MZL, lymphoplasmacytic lymphoma and rarely in follicular lymphoma.

The Cytometry Society-India
15th Annual Conference & Workshops
26th -29th October, 2023
Theme: From Basics to Multi-Omics

C3 - Identification of heterogenous markers (CD48, CD52, CD96, CD88) that define Leukemic Stem Cells and Hematopoietic Stem Cells in AML by flow cytometry

Aafreen Khan, Deepshi Thakral, Ritu Gupta, Vivek Kumar Singh, Sandeep Rai, Vijay Kumar, Sameer Bakhshi

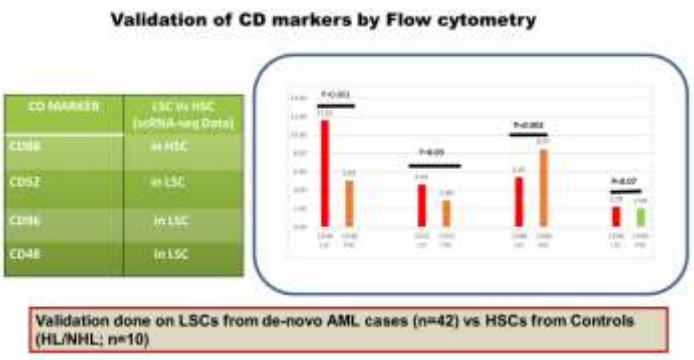
AIIMS, DELHI

Introduction: AML is a highly heterogenous disease that is sustained by a minor population of cells namely Leukemic Stem Cells (LSCs) with an ability of self-renewal and reinitiation of leukemia. These LSCs pose a major obstacle in AML treatment with higher rates of induction failure (refractory AML) and relapse. Moreover, discerning the mechanisms of chemoresistance is hampered by lack of definitive LSC markers. Therefore, markers that might facilitate the identification of LSC fraction from patient samples and differentiate these from normal HSCs (Hematopoietic Stem Cells) in AML need to be investigated.

Aims & Objectives: In this study, we aimed to identify novel markers to differentiate between leukemic stem cells (LSCs) and normal hematopoietic stem cells (HSCs) in acute myeloid leukemia (AML).

Materials and methods: Using single-cell transcriptome-based analysis (n=18) and flow cytometry (n=42) using a 14-color antibody panel, we investigated the expression patterns of CD52, CD48, CD96 as potential markers for LSCs, and CD88 as a marker for HSCs within the progenitor cell compartment defined by CD34 and CD38. The pattern and intensity of expression of CD52, CD48, CD96 and CD88 (C5AR1) were evaluated in comparison to progenitor cell compartments in healthy bone marrow samples using the median fluorescence intensity (MFI) values.

Results: From scRNA-seq analysis, we observed a consistent upregulation of CD48, CD52 and CD96 transcript expression in LSC populations as compared to HSC populations. The expression of these markers was further validated by flow cytometry analysis of CD34+CD38- enriched LSC fractions from AML patient samples. The expression levels correlated with MFI values of as determined by flow cytometry and found to be significantly higher in LSCs, indicating that CD48, CD52 and CD96 could be valuable markers for identifying and characterizing LSCs in AML.



Conclusion: CD48, CD52, CD96 are potential LSC enrichment markers while CD88 is highly expressed on normal HSCs. This study warrants further investigation for targeting these potential LSC markers to improve clinical outcomes in patients.

C4 - Establishing the Limit of Blank (LOB), Limit of Detection (LOD), and Lower Limit of Quantitation (LLOQ) of a 16-color multicolor flow cytometry (MFC) based measurable residual disease assay across genetic/phenotypic subtypes of Acute Myeloid Leukemia

Sitaram Ghogale, Jagruti Patil, Simpy Raj, Priyanka Dhende, Nilesh Deshpande, Karishma Girase, Gaurav Chatterjee, Sweta Rajpal, Nikhil Patkar, Sumeet Gujral, P. G. Subramanian, Prashant Tembhare
TATA MEMORIAL CENTRE- ACTREC

Background: Determining LOB/LOD/LLOQ is essential to a quantification-sensitive test such as MFC-MRD. A key factor for LOB/LOD/LLOQ is the reproducible immunophenotype of blasts. However, AML is a group of genetically diverse diseases with considerable immunophenotypic heterogeneity which offers challenges in establishing LOB/LOD/LLOQ in AML. However, AML with a homogeneous genetic abnormality often demonstrates consistent immunophenotype. We established LOB/LOD/LLOQ for various genetic/immunophenotypic subgroups of AML using a 16-color MFC-AMLMRD assay.

Methods: We developed a 16-color MFC-AMLMRD assay using bulk-lysis, acquisition on an LSR-Fortessa followed by analysis on Kaluza v2.1. LOD/LLOQ assay was performed in triplicate on AML samples using spiking and dilution experiments with normal BM cells to obtain an expected level of 30–1000 leukemic blasts in 1–5 million non-leukemic cells (translating into 0.001%/0.005%/0.01%/0.1%/1%). Separate experiments were performed for AML with RUNX1::RUNX1T1 (CD34+CD56+CD19+blasts – AML-S1), AML with NPM1 (CD34-CD117+blasts– AML-S2), AML with KMT2A-rearrangement (CD34-CD117- CD33+CD13+HLADR+blasts– AML-S3), AML with CEBPA (CD34+CD117+CD7+blasts – AML-S4), AML with FLT3-ITD (CD34+CD117+CD123+blasts– AML-S5) and AML-M7 with NUP98-rearrengment (CD34-CD42b+CD36+CD41+CD61+HLADR+blasts– AML-S6).

Results: These experiments showed variable results across subgroups (S1-S6) of AML. LOB of AML-S1/S2/S3/S4/S5/S6 were 30/25/350/60/20/40 events respectively. LOD of AML- S1/S2/S3/S4/S5/S6 were 50/40/600/100/33/65 events respectively. LLOQ of AML-S1/S2/S3/S4/S5/S6 were 94/40/600/100/33/65 events respectively. A CV<10% at this level was reached for all subgroups of AML at level of 0.001% except AML-monocytic differentiation (i.e. KMT2A-rearrangement) in which CV<10% was observed at 0.04%). Thus, we determined a LOD of 0.005% (range, 0.001-0.005) and LLOQ of 0.007% (range, 0.001-0.007) across six subgroups of AML using 16-color AMLMRD assay.
`

Conclusion: Our results demonstrated the variability in establishing LOB/LOD/LLOQ for a MFC-AMLMRD assay across various subgroups of AML highlighting its challenges in real- world applicability. We further showed that MFC-AMLMRD requires individual LOB/LOD/LLOQ assessment for each subgroup of AML.

C5 - FLAER as a Standalone Diagnostic Reagent in Paroxysmal Nocturnal Hemoglobinuria Testing: Do We Need to Rethink Guidelines?

Praveen Sharma1*, Parveen Bose1*, Nabhajit Mallik1, Dikshat Gopal Gupta2, Suneel Rachagiri1, Arun Kumar1, Jasbir Kaur1, Pankaj Malhotra3, Neelam Varma1, Man Updesh Singh Sachdeva1#

1.Department of Hematology, Post Graduate Institute of Medical Education and Research, Chandigarh, 160012, India.

2.Department of Urology, The Robert H. Lurie Comprehensive Cancer Center, Northwestern University Feinberg School of Medicine, Chicago, Illinois, 60611, United States of America (USA).

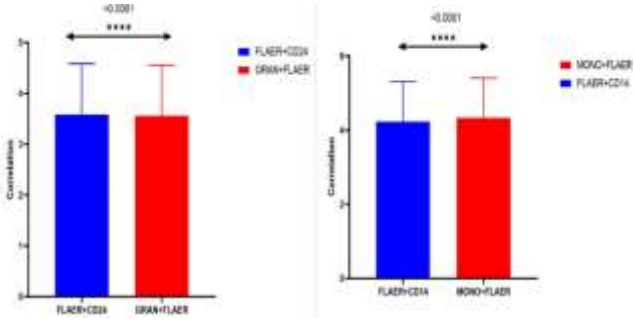
3.Department of Clinical Hematology & Medical Oncology, Post Graduate Institute of Medical Education and Research, Chandigarh, 160012, India.

Introduction: FLAER's ability to bind to a wide array of GPI-linked structures across various leukocyte subsets is remarkable. We hypothesize that employing FLAER as the sole reagent may be just as effective in detecting PNH clones. This study aims to compare the results obtained from a FLAER-only approach to the recommended FLAER+GPI-linked protein strategy, with a focus on its applicability in clinical settings.

Methods: Two distinct panels were utilized: the "routine panel," which included gating markers (CD45 for WBCs, CD15 for granulocytes, and CD64 for monocytes) in addition to FLAER and GPI-linked markers (CD24 and CD14); and the "FLAER-only panel," consisting of the gating markers and FLAER alone (excluding CD24 and CD14). EDTA-anticoagulated samples were processed by the lyse-wash-stain-wash technique, with data acquisition performed on a BC Navios Ex flow cytometer (Beckman Coulter Inc, USA) and subsequent analysis on Kaluza Software 2.1. LOB, LOD and LLOQ were also determined.

Results: A total of 209 patients were subjected to testing. Both panels identified PNH clones in 20.1% of patients (n=42/209) with a 100% concordance rate. The PNH clone range for granulocytes in the routine panel was 0.01-89.68%, and for monocytes, it ranged from 0.04-96.09%. The FLAER-only panel showed a granulocyte PNH clone range of 0.01-89.61% and a monocyte range of 0.01-96.05%. Pearson correlation statistics (figure 1) demonstrated a significant correlation between the size of PNH clones in granulocytes and monocytes for both panels (granulocytes r=0.9999, p<0.0001, 95% CI=0.9999 to 1.000; monocytes r=0.9974, p<0.0001, 95% CI=0.9966-0.9980).

Conclusion: Our findings support the efficacy of FLAER as a standalone marker, showcasing its specificity and sensitivity in identifying PNH clones within granulocytes and monocytes, even in high-sensitivity PNH assays. The proposed "FLAER-only panel" emerges as an efficient and cost-effective solution for highly sensitive PNH testing across two distinct cell lineages for application in clinical settings.

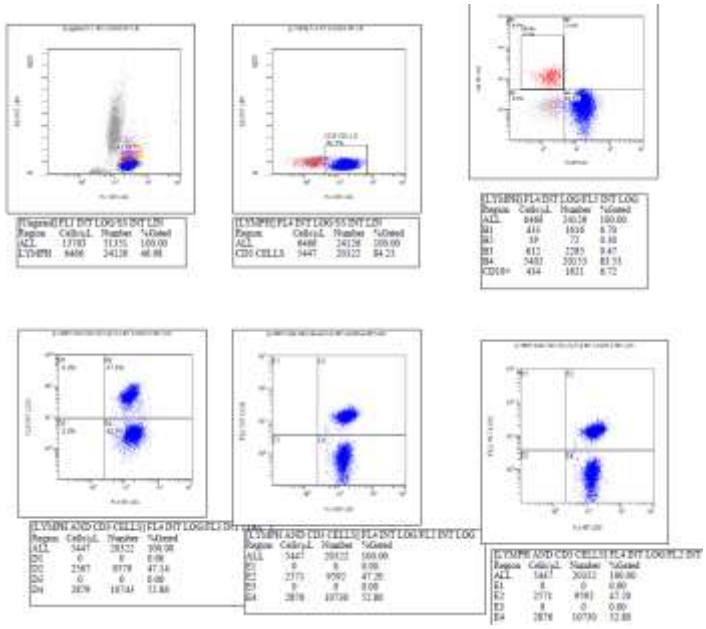


Abstracts - Poster Papers Clinical

CP1 - Flowcytometric assessment of blood lymphocyte subpopulation in pediatric patients of Steroid sensitive and resistant Idiopathic Nephrotic Syndrome

Dr. Gautom Kumar Saharia, Dr. Anju Ashok AK, Dr. Amit Satapathy, Dr. Manaswini Mangaraj
AIIMS, Bhubaneswar

Idiopathic Nephrotic syndrome (INS) constitutes most cases of Nephrotic Syndrome in children. It is detected in about 9-16 children every year per 1 lakh children in Asia as compared to a global rate of 2-7 cases per 1 lakh children per year. Males are more affected in comparison to females at the ratio of 3:1. They are either of Steroid sensitive nephrotic syndrome (SSNS) or Steroid resistant nephrotic syndrome (SRNS) type. SSNS cases continue to have relapses in adulthood having <5% risk of future chronic kidney disease and SRNS cases possess an enhanced risk of progression to End Stage Renal Disease (ESRD), their early detection with an understanding of pathophysiology is needed to prevent future comorbidities like infection, thrombophlebitis etc. and side effects of prolonged steroid therapy. With this background, the study is undertaken with the objectives to detect the alteration of blood lymphocyte subpopulation in INS cases with CD3, CD45, CD4, CD8 and CD19 assay in comparison to healthy controls and to assess variations in blood lymphocyte subpopulation in response to steroid therapy. A total of 34 Cases with a confirmed diagnosis of Idiopathic Nephrotic Syndrome attending Pediatric OPD were recruited and divided into 2 subgroups based on response to standard steroid therapy of 2 mg/kg/day. The cases were followed up over a period of 4 weeks after the commencement of steroid therapy. 17 normal children attending the Pediatric OPD for monitoring growth or for vaccination purpose were taken as controls. In addition to the routine baseline investigations, whole blood was collected to conduct analysis of CD markers like CD3, CD4, CD8 and CD 19 to identify the variations in the count of B lymphocytes and T lymphocytes with the disease progression after 4 weeks of steroid therapy. Mean age of the participants were 5.76± 0.82 years. The mean cell count of the SSNS group on 1st visit for CD3, CD4, CD8, CD19 were found to be similar with control group but lower than SRNS group. The subpopulation counts on follow up visit were much higher for both the groups. The precise cause of INS remains elusive which is best defined as a Podocytopathy. Systemic factors (i.e., immune mediated or circulating mediators) are the major contributors to this podocyte effacement. Dysregulation or dysfunction of T lymphocytes are supposed to be involved in its pathogenesis.



CP2 - Adult T cell leukemia/lymphoma with varying clinical and immunophenotypic presentations from a non-endemic region for HTLV-1 in South India

Dr. Ansu Abu Alex, Dr. Mobin Paul, Dr.Latha Abraham
Rajagiri Hospital, Kochi, Kerala

Background and Introduction : Adult T cell leukemia/lymphoma (ATL), one of the most aggressive among the peripheral T cell lymphomas, is a malignancy of mature CD4+ T cells, caused by the oncogenic retrovirus, human T-cell leukemia virus type 1 (HTLV-1).We have case reports of two patients with ATL presented to our hospital in a span of one year with different clinical and phenotypic presentations. Methods and Results:

Case 1 was a 56 year old female diagnosed outside as lymphomatoid papulosis. Her blood investigations showed a total count of 7700*103/ul, Hb of 10.6 g/dl with elevated ESR 50 mm/hr. Peripheral smear shows 10% atypical cells and skin biopsy revealed mature T cell neoplasm. Bone marrow aspiration showed 3% atypical lymphoid cells and flow cytometric analysis showed 14% CD3 dim population (on lymphocytes) positive for CD2, CD5 , TCRab, CD4+CD25+, CD8 -ve and CD7 (dim to negative) (Fig1). HTLV1/2 antibodies was strongly reactive and her diagnosis was confirmed as ATL- smouldering type.

Case 2 was a 69 year old male with generalised tiredness and has total count 75.1 *103/ul with 79% lymphocytes, elevated LDH (1682 U/L), creatinine (2.3mg/dl), hypercalcemia(19.34mg/dl) and hyper uricemia(10.7mg/dl). Peripheral smear shows 79 % atypical lymphoid cells with markedly intended flower shaped nuclei. Bone marrow showed 32% atypical lymphoid cells and immunophenotyping showed 47% CD45 bright population with CD5+, CD3+, CD4+CD25+, TCRab+ CD8-ve, CD7-ve (Fig1). HTLV1/2 antibodies was strongly reactive and a diagnosis of ATL - acute type was confirmed.

Both patients were treated with Zidovudine+interferon alpha 2 B, but Case1 lost to follow up after 9 months and other patient survived only for 3 months. Conclusion Even though a non endemic region for HTLV-1, there are a few reports of ATL from Kerala and other parts of India. Because of the extreme clinicopathologic heterogeneity, accurate identification requires combination of clinical features, morphology and immunophenotypic findings along with positive HTLV1/2 serology.

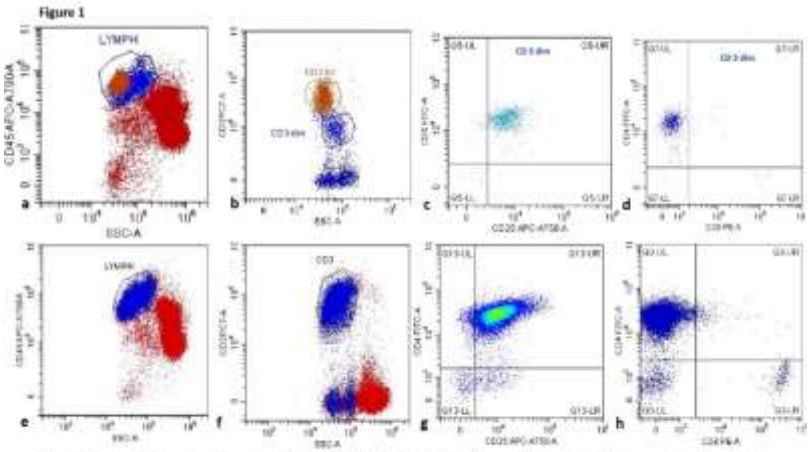


Figure 1. Representative flow cytometry plots of case 1 with CD3 bright and dim population and CD3 dim population shows CD4+ CD25+, CD8-ve and CD7 dim to negative (a-d) and Case 2 with CD3 bright population showing CD4+CD25+ (bri) with CD8 and CD7 negative (e-h).

CP3 -Flow cytometric evaluation of normal ranges of surface immunoglobulin B-cell subsets in healthy controls and its clinical significance

AMRUTA JAGDISH ACHARYA, Priyanka Dhende,Aastha karla,sukriti takur, Harshini Sriram,Sitaram Ghogale, Nilesh Deshpande, Karishma Girase, Jagruti Patil, Gaurav Chatterjee, Nikhil Patkar, Sumeet Gujral,P. G. Subramanian,Sweta Rajpal, Prashant Tembhare

TATA MEMORIAL CENTRE (ACTREC)

Introduction: Common variable immunodeficiency (CVID) is a rare inborn errors of immunity (IEI) characterised by significantly reduced immunoglobulin levels. Recently, Euroflow has demonstrated the utility of B-cell subset levels expressing different surface heavy-chain immunoglobulins (sIg) in the diagnosis of IEI. However, data on the normal range of sIg+B-subsets in healthy controls is limited and there is no data from Indian population. Hence, we are using flow cytometer to valuate normal ranges of sIg subsets in healthy controls.

Methods: We studied sIg+B-subsets in peripheral blood of 36 healthy volunteers.11-color flow cytometry assay with antibodies against IgG1 (clone SAG1), IgG2 (clone SAG2), IgG3 (clone SAG3), IgG4 (clone SAG4) IgA1 (clone SAA1), IgA2 (clone SAA2), IgM (clone, G0-127),IgD (clone IA6-2) along with backbone B -cell. Data was acquired on LSR Fortessa (BD Biosciences) and analysed using Kaluza software version2.2.

Results: Median age of volunteers was 27 years (range 22-45 years). The median percentage of B-lymphocytes was 4.95% (range 1.89-18.62). Median (range) of naive and memory B-cells were 70.65% (43.67-86.14) and 25.19% (5.02-53.09) respectively. The median percentage of IgG1+B was 5.78% (range 0.76-15.33), IgG2+B 2.16% (range 0.35-5.63), IgG3+B 1.35% (range 0.10-8.36), IgG4+B 0.31% (range 0.02-1.17), IgA1+B 3.44% (range 0.29-7.73), IgA2+B 1.65% (range 0.20-6.13), IgM+B 84.20% (range 64.32-93.99) and IgD+B 79.18% (range 56.66-96.30). Using these levels, we have diagnosed CVID case which shows 97.30% of all B-cells expressing IgD and 94.05% B-cells expressing IgM. IgG+/IgA+ memory-B-cells were markedly reduced. B-cells also showed moderate positivity for CD21 but CD81 and CD23 were negative.

Conclusion: We have established a normal range of various surface immunoglobulin expressing B-cell subsets in healthy volunteers and demonstrated its clinical utility. These ranges are useful to enhance the utility of flow cytometric diagnosis inborn errors of immunity including CVID.

CP4 -Flow Cytometric based Immunophenotyping of Acute Lymphoblastic Leukemia in Adults: An Experience from Single Institution

Sridevi HB, Akshaya.S, Vatsala KB

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

Introduction: Acute lymphoblastic leukemia(ALL) is the second most common leukemia in adults. Although 80% of ALL occurs in children, it has poorer prognosis in adults. Immunophenotype and cytogenetic evaluations are the objective tests in its accurate diagnosis and subtyping.

Objectives: To study the clinicopathologic findings and evaluate the immunophenotypc features of adult ALL.

Methodology: A retrospective study of 8 years duration was conducted in a tertiary care referral center where all immunophenotypically diagnosed cases of ALL in adults were included.Clinical findings,peripheral blood cell count at presentation, morphological and cytochemical evaluation of blasts in blood or marrow aspirate were evaluated. Spectrum of immunophenotypic features in subtyping ALL were assessed along with aberrant expression by the blasts.

Results: This study included 96cases, with a male predominance(M:F-1.7:1) and a mean age of 40.2years.Clinically,most common presentations were organomegaly and fever. Mediastinal mass was observed in few cases of T-ALL. T-ALL presented with highter leucocyte count and in younger population than B-ALL.Morphologically, cleaved cells were seen in T-ALL, although deeply clefted cells were also observed in B-ALL.On immunophenotyping, with expression of specific antigens in variable proportion, 75cases(78.1%) were B-ALL and 21cases(21.8%) were T-ALL. Cytoplasmic CD79a was expressed in 28cases of T-ALL. A case of pre-thymic cytoplasmic CD3 negative T-ALL was encountered.Aberrant myeloid expression was noted in 13.5% cases with a good distribution of cases having aberrancies in both B-cell and T-cell types.

Conclusion: Adult ALL is not an uncommon disease.Morphological and cytochemistry evaluation aids in guidance,however,immunophenotyping is an essential and integral part of diagnosis and management of ALL. Adult ALLs are classified on immunophenotyping with pre-B ALL being a common phenotype.Immunophenotyping can not only confirms the diagnosis of acute leukemia,but also can provide additional findings of subtyping, picking up cases of mixed phenotypic leukemia,identifying rarer subtypes of ALL and the various prognostic factors and thus has a pivotal role.

CP5 -Flow Cytometric evaluation of CD38 expression in T-cell Non-Hodgkin Lymphoma (T-NHL) and its implication for anti-CD38 targeted therapy

SHRADDHA SURESH BANE, Aastha karla,Harshini Sriram,Sitaram Ghogale, Nilesh Deshpande, Karishma Girase, Jagruti Patil, Gaurav Chatterjee, Nikhil Patkar, Sumeet Gujral,P. G. Subramanian,Sweta Rajpal, Prashant Tembhare

TATA MEMORIAL CENTRE- ACTREC

Introduction:T-NHL is an aggressive, heterogeneous group of lymphomas with poor prognosis. No therapy provides long-lasting response, hence a need for targeted therapeutic approaches. Anti-CD38 monoclonal antibody (antiCD38-Mab) has become a promising targeted therapy in many hematological malignancies. Its anti-tumor efficiency depends on level of CD38 expression on abnormal cells. Therefore, we evaluated CD38expression in tumor cells from T-NHL patients.

Methods: Expression pattern (percentage and mean fluorescent intensity, MFI) of CD38 on atypical lymphocytes (ATLs) was studied in 50 T-NHL patients by Multicolor Flow Cytometry (MFC). Data was acquired on10-13 color CytoFLEX (Beckman Coulter), and analyzed using Kaluza-v2.1 software. Normalized MFI (nMFI) was determined as: nMFI = (Tumor MFI-NC MFI) *10/ (PC MFI- NC MFI)

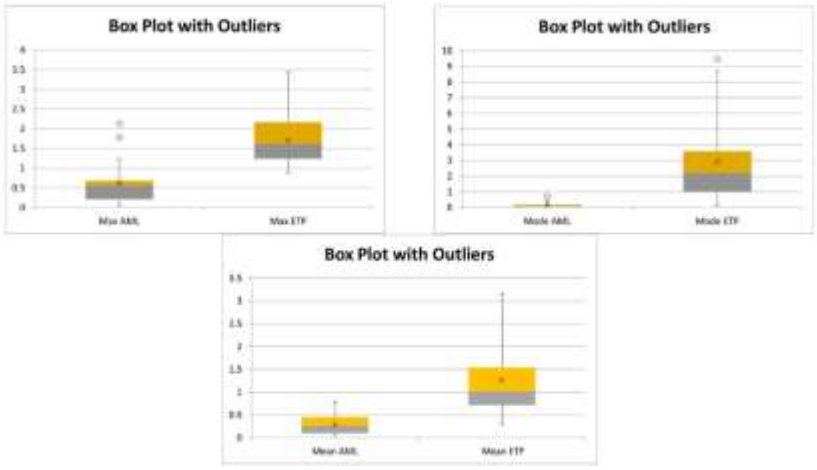
Results: Out of 50 T-NHL (median age- 49 years; range- 1-80 years), 23(46%) were Angioimmunoblastic T-cell Lymphoma (AITL), 6(12%) Anaplastic Large Cell Lymphoma (ALCL), 6(12%) Gamma-Delta T-cell Lymphoma (GD-TCL), 2(04%) T-Lymphoblastic Lymphoma (T-LBL), 2(4%) T-cell Large Granular Lymphocytic leukemia (T-LGLL), 2(4%) T/NK-Non Hodgkin Lymphoma (T/NK-NHL), 2(4%) Cutaneous T-cell Lymphoma (CTCL),1(2%)T-cell Prolymphocytic Lymphoma (T-PLL) and 6(12%) Peripheral T-cell Lymphoma–NOS (PTCL-NOS). Median age was 49 (range 1-80) years. NK-cells were taken as internal positive control and granulocytes as internal negative control, the median MFI of which were 8.12(range 1.71-17.35) and 1.41(range 0.10-4.41) respectively. The nMFI and percentage of CD38 expression in T-NHLs were1.98(range 0.0-15.74) and 43.59%(range 0.54-99.19%)respectively. CD38 was homogeneously expressed in 11/23(47.8%) AITL, 3/6(50%) GD-TCL, 2/6(33%) ALCL, 2/2(100%) T-LBL, 2/2(100%) T/NK-NHL, 1/2(50%) T-LGLL, 1/1(100%) of T-PLL, 0/2(00%) CTCL and 2/6(33%) PTCL- NOS.

Conclusion: We report the expression pattern and levels of CD38 expression in patients with T-NHL. Our data showed that CD38 was homogenously expressed in 48% of all T-NHL studied. It was robustly expressed in 48% cases of AITL and 50% cases of GD-TCL making it potentially eligible for antiCD38-Mab therapy

CP6 -Reliability of CD7 intensity as a differentiator between Early T cell Precursor ALL and CD7 positive Acute Myeloid Leukemia

ANANTHVIKAS JAYARAM, PRADEEP KUMAR V, SYAM KUMAR M S, SABAN KUMAR CHOUDHARY
NEUBERG ANAND REFERENCE LABORATORY, BANGALORE

Background Cytoplasmic CD3 expression is a key determinant in the differential diagnosis of Early T cell precursor Acute Lymphoblastic Leukemia (ETP-ALL) from Acute Myeloid Leukemia expressing CD7 (CD7+AML). Often is the case that CD3 intensity in ETP-ALL is weak, and the ambiguity in interpretation of the standard recommendations for cytoplasmic CD3 leads to difficulty in diagnosis.
Objective To assess if intensity of CD7 expression can be useful in differentiating ETP-ALL from CD7+AML
Methods Flow cytometry data obtained on a BD FACSLyric 12 colour flow cytometer from 20 sequential CD7+AML cases and 16 sequential ETP-ALL cases were analysed on a BDFACSSuite platform. The mean, mode, and maximum fluorescence of CD7 was noted in the gated blast population and Natural Killer cells (CD7+ CD3- lymphocytes). A ratio of blasts/NK cells was then calculated for each of these parameters. Statistical parameters were calculated on Microsoft excel using statistical analysis package plugins.
Results All three parameters showed a significantly higher value in the ETP-ALL subgroup when compared to the CD7+AML group ($p<0.05$). Using ROC curve analysis, the following cut-off values were obtained with optimal sensitivity and specificity for identifying ETP-ALL. Cut off Sensitivity Specificity P value (T test) Mode 0.3 93.3% 90% 0.0027 Mean 0.6 85.7% 95% 0.0003 Maximum 0.8 100% 80% 0.00004 Discussion: A higher intensity of CD7 proved to be a useful adjunct to Cytoplasmic CD3 expression in identifying ETP-ALL in our series and improved our confidence especially in those cases with weak expression of CD3. Addition of this information to the diagnostic algorithm may help alleviate some of the ambiguity surrounding interpretation of cytoplasmic CD3 in ETP-ALL.



CP7 -EX-VIVO GRAFT MANIPULATION WITH FOR PEDIATRIC HAPLOIDENTICAL HSCT: A FLOW CYTOMETRIC ANALYSIS FOR QUALITY CONTROL AND EFFICACY OF MANIPULATION

Ankit Malhotra, Divya Setya, Satyendra Katewa, Rahul Sharma, Ravi Dara, Monica Singh, Surendra Sharma
Manipal Hospitals

Background: To avoid graft-versus-host disease (GvHD) associated with haploidentical hematopoietic stem cell transplantation (HSCT), many graft manipulation strategies are available including enrichment and depletion. TCRαβ + T cell and CD19 + B cell depletion is a graft manipulation method for haploidentical (HSCT) retaining hematopoietic stem cells and TCRγδ+T cells. Aim of the present study was to assess the composition of the graft for pediatric haploidentical HSCT after manipulation.

Materials and Methods: A retrospective study to assess the efficacy of graft manipulation was performed in the department of Hematopathology and Molecular Biology at a tertiary healthcare facility in North India from 2017 to 2022. All peripheral blood hematopoeitic stem cell products collected using Com.Tec (Fresenius Kabi, Germany) cell separator which underwent cell processing in CliniMACS (Miltenyi Biotech, USA) and flowcytometry based assessment of graft composition and viability pre and post manipulation processing for pediatric haploidentical HSCT to treat hematological malignancies were included in the study.

Results: A total of 5 hematopoeitic stem cell grafts collected for pediatric hematological malignant disorders, were manipulated during the study duration. Mean CD34 + cell dose/kg collected by apheresis was 26.89million with a mean viability of 98%. Mean log reduction of TCRαβ + T cells and CD19 + B cells was 3.9 and 3.3 respectively. No significant changes were observed in viability with processing. Cell recovery was 81.2% for CD34+ hematopoietic stem cells and 75.9% for TCRγδ+T cells.

Conclusion: A desirable stem cell and TCRγδ+T cells recovery and effective depletion of TCRαβ+T cell and CD19 + B cells was observed with graft manipulation which is favourable for improved transplant outcomes in pediatric haploidentical HSCT performed for hematological malignancies. A thorough quality control (QC) of the apheresis product by flowcytometry helps in assessing the impact of manipulation on the graft.

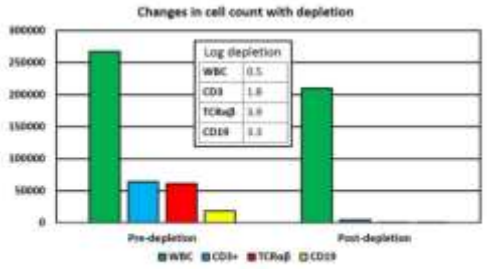


Figure 1: Mean cell counts before and after graft manipulation

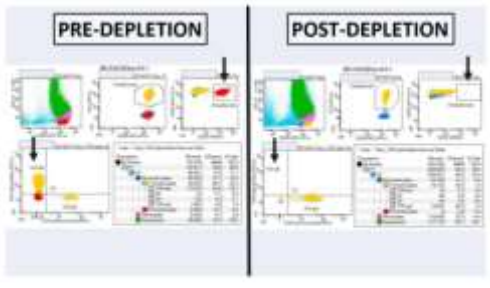


Figure 2: Flowcytometric analysis of graft composition before and after manipulation

CP8 -Utility of Flow Cytometry Immunophenotyping in peripheral blood monocytes for the Diagnosis of Chronic Myelomonocytic Leukemia (CMML)

KOMAL KAMLESH GULABRAI,Priyanka Dhende, Maithili Dalvi, Harshini Sriram, Sitaram Ghogale, Nilesh Deshpande, Karishma Girase, Jagruti Patil, Gaurav Chatterjee, Nikhil Patkar, Sumeet Gujral,P. G. Subramanian,Sweta Rajpal, Prashant Tembhare

TATA MEMORIAL CENTRE- ACTREC

Introduction: Chronic myelomonocytic leukemia (CMML) is a rare hematologic malignancy of elderly people with a high risk to progress to acute myeloid leukemia (AML). Majority of cases show morphological and genetic features overlapping with either reactive monocytosis or myelodysplastic syndromes/myeloproliferative neoplasms (MDS/MPN).However, there is no data from India. Hence, we are studied the utility of FCI and applicability of recently published criteria in the diagnosis of CML.

Methods: We studied FCI features in peripheral blood (PB) and bone marrow (BM) samples from 15 patients of clinically and morphologically suspicious of CMML using 10-16-color antibodies panel- CD64/ CD56/ CD14/ CD33/ CD16/ CD300e/ HLADR/ CD36/ CD15/ CD34/ CD38/ CD117/ CD13 /CD177 /CD45 /CD11b. Data was acquired on LSR-Fortessa (BD Biosciences), analysed using Kaluza software version2.2. Cytogenetic and molecular findings by NGS were noted. PB-FCI was focused on measurement of monocytes subset-classical-MO1 (CD14+/CD16–), intermediate MO2 (CD14+/CD16+) and non-classical MO3 (CD14−/CD16+).

Results: The study included 15 patients with median age- 64years; (range 28-88 years) and M: F-4:3. Among 15 cases, median percentage of MO1, MO2 and MO3 in PB were 90.6%,6.9%,0.9% respectively. These findings showed that MO1-monocytes were ≥94% in 33.33% patients which is similar to recently published reports. We also noted immunophenotypically aberrant maturation patterns and other abnormalities in BM samples. Of 15 patient's abnormal blasts noted in 9/15(60%), 10/15(66.6%) abnormal granulocytic maturation patterns, and 8/15(53.3%) abnormal monocytic maturation patterns. Cytogenetic data was available in 7/15 cases of which 2 cases showed for Trisomy 8 and 1 del 20q while 4 cases no cytogenetic abnormality detected. NGS data available in 7/15 cases of which 4 cases showed abnormal mutation profile like RUNX1, CBL, NRAS, TET2, CEBPA and 3 cases were neg.

Conclusion: Our study showed the clinical utility of PB-FCI and BM-FCI findings in the diagnosis of CMML and also the easy applicability of FCI-criteria for the diagnosis of CMML which is a rare and challenging neoplasm.

CP9 -Flow Cytometric evaluation of CD37 expression in B-cell Non-Hodgkin Lymphoma (B- NHL) and its implication for anti-CD37 targeted therapy

Nisha Narendra Jamadar, Priyanka Dhende, Harshini Sriram,Sitaram Ghogale, Nilesh Deshpande, Karishma Girase, Jagruti Patil, Gaurav Chatterjee, Nikhil Patkar, Sumeet Gujral,P. G. Subramanian,Sweta Rajpal, Prashant Tembhare

Tata Memorial Centre (ACTREC)

Introduction: B-NHL is a heterogeneous group of lymphomas with differing patterns of behavior and responses to treatment. With current therapy, many patients eventually relapse or experience resistance creating need for new targeted therapies. Anti-CD37 monoclonal antibody (antiCD37-Mab) therapy is upcoming as a new targeted therapy in B-NHL. Its anti- tumor efficiency depends on the level of CD37 expression on abnormal cells. Hence, we evaluated CD37 expression levels in tumor cells from B-NHL patients.

Methods: Expression pattern (percentage and mean fluorescent intensity (MFI)) of CD37 on atypical lymphocytes (ATLs) was studied in 26 uninvolved staging marrows and 44 B-NHL patients sample for diagnosis or staging using Multicolor Flow Cytometry (MFC). MFC was performed on 16 color LSR Fortessa BD and data was analyzed using Kaluza-v2.2 software.

Results: Out of 44 B-NHL cases (median age-59.5years; range- 30-98 years), 26(59.09%) were Chronic lymphocytic leukemia (CLL), 5(11.3%) follicular lymphoma (FL), 5(11.36%) mantle cell lymphoma (MCL), 3(6.8%) each of Marginal zone lymphoma (MZL), 3(6.8%) Hairy cell leukemia (HCL) and 1(2.2%) lymphoplasmacytic lymphoma (LPL), 1(2.2%) Diffuse large B cell lymphoma (DLBCL). In uninvolved staging marrows CD37 was moderately expressed in all mature B lymphocytes with median MFI of 76.94(range 18.4-250.5) and median coefficient of variation (CV) of 63.71(range 47.95-166.9). In T lymphocytes, granulocytes and monocytes CD37 was negative. The median MFI, percentage and CV of CD37 expression in B-NHLs were 65.3%(range 30.4- 194.14), 98.56%(range 81.38-99.96%) and 56.97(range 39.98-150.3) respectively. The median percentage of CD37 positive abnormal B-lymphocytes in CLL were 98.15%(range 80.95-99.96 %), FL 97.64%(90.51-98.56 range %), MCL 97.64%(81.38-99.79 range %), 98.15 MZL %(99.58- 99.79 range %), HCL 98.85%(99.11-99.87range %), LPL 97.45% and DLBCL 99.47.

Conclusion: We report the expression pattern and levels of CD37 expression in patients with B-NHL. Our data showed that CD37 was homogeneously expressed in all B-NHLs studied making it potentially eligible for antiCD37-Mab therapy

CP10 -Assessing the Cytotoxic Effects of Methyl Methanesulfonate (MMS) on Human Lymphocytes: Implications for Cancer Biology and Diagnosis

Mohd Mustafa, Safia Habib, Asif Ali, Shahid Ali Siddiqui, Moinuddin

ALIGARH MUSLIM UNIVERSITY

Introduction: Hematology, the study of blood and blood-related disorders, holds a pivotal role in understanding and treating a wide range of medical conditions. In this context, this research delves into the impact of Methyl methanesulfonate (MMS) on human lymphocytes and its potential significance in cancer biology and diagnosis.

Objective of the study: To study the toxicity of MMS on human peripheral lymphocytes. Materials and methods: Experimental procedures involved an array of analytical techniques, including ROS assessment, MMT assay, % cell viability, apoptosis analysis, and IC50 assays on lymphocytes. Morphological changes were evaluated using scanning and confocal microscopy.

Results and discussion: The results revealed that MMS inflicted significant harm on cells, leading to DNA damage and reduced lymphocyte viability, exacerbating reactive oxygen species (ROS) levels and prompting apoptosis. Microscopic analyses further confirmed these adverse effects.

Conclusion: Chronic exposure to MMS may result in altered morphology of lymphocytes, and if the condition persists may impair the immune status of the individual. Understanding the pathophysiology and molecular mechanism associated with MMS toxicity and lymphocyte DNA damage may pave the way for the early diagnosis of inflammatory carcinogenesis. Keywords: Methyl methanesulfonate, lymphocytes, toxicity, cancer diagnosis.

CP11 -Early T Precursor Acute Lymphoblastic Leukemia -case report

Dr.J.Latha Fathima,Dr.Parimala P, Dr. Shanthala Devi AM
St. Johns National Academy of Health Sciences

Background: Early T-cell precursor acute lymphoblastic leukemia (ETPALL) is a distinct, rare subtype of T-ALL. The blasts in ETPALL retain both the lymphoid and myeloid differentiation, indicating it is directly derived from hematopoietic stem cells. Case report The presentation and features of 3 patients diagnosed with Early T-ALL are discussed.

Case 1. A 54-year-old male patient with complaints of fever was evaluated. Peripheral blood smear showed few atypical lymphoid cells. Bone marrow aspirate showed sheets of blasts (96%). Immunophenotyping showed features of early T ALL. Induction phase I was completed and the patient was admitted within a month with relapse.

Case 2. A 41-year-old male with tiredness, cough, and fever for 3 months was evaluated. Peripheral smear examination revealed marked leukopenia. Bone marrow aspirate showed 65% blasts. Immunophenotyping revealed early T-ALL. The patient had completed Phase 2 induction and is on follow-up.

Case 3. A 19-year-old male was admitted with complaints of headache. Peripheral blood smear showed 85% blasts. Immunophenotyping was done in peripheral blood and found to be a case of early T ALL. The immunophenotyping of the 3 cases are tabulated. Precursor marker Myeloid B markers T markers CD34 HLA-DR TDT CD 117 MPO CD13 CD33 CD19 CD10 CD79b CD3(cytoplasmic) CD5 CD7 Patient 1 Pos Neg Neg Pos Neg Pos Pos Neg Neg Neg Pos Neg Pos Patient 2 Pos Pos Neg Neg Neg Neg Pos Neg Neg Neg Pos Neg Pos Patient 3 Pos Neg Neg Pos Neg Pos Pos Neg Neg Neg Pos Neg Pos

Case history

Case details	Patient 1	Patient 2	Patient 3
Age	52	41	19
Sex	Male	Male	Male
Symptoms	Fever, breathlessness	Tiredness	Headache
Sign	Pallor and pitting edema No lymphadenopathy	Pallor No lymphadenopathy	Pallor
Peripheral blood examination	Atypical lymphoid cells present (11%)	Marked leukopenia 1.0d x 10*3cells/ul	Leukopenia with 85% blasts
Bone marrow aspirate	Hypercellular marrow with 96% blasts	Hypercellular bone marrow with 65% blasts	Not done
Immunophenotyping by flow cytometry	Positive for CD 34, TDT, CD117, CD13, CD 3(Cytoplasmic), CD5, CD7, Negative for HLA-DR, MPO, CD13, CD14, CD64	Positive for CD34, HLA-DR, CD33, CD3(Cytoplasmic),CD7 Negative for TDT, MPO, CD13, CD117,CD19,CD10,CD20 CD5	Positive for CD 34, CD 117, CD13, CD33, CD 3(Cytoplasmic) CD7 Negative for TDT, HLA DR, MPO,CD19, CD10,CD20
Treatment	Completed Induction Phase I, readmitted with fever	Completed phase II induction, on follow up	Completed Induction , patient is on follow up

Conclusion: The striking immunophenotypic signature of ETP-ALL differentiates from other types of ALL and mixed phenotype acute leukemia. The diagnosis of ETPALL is important because of poor outcomes and high relapse rate. Keywords-Early T Precursor ALL, blasts

The Cytometry Society-India
15th Annual Conference & Workshops
26th -29th October, 2023
Theme: From Basics to Multi-Omics

CP12 -Evaluation of monitoring B cell counts in Nephrotic Syndrome management

Vanamala A Alwar, Shanthala Devi AM, Parimala P, Latha JF, Sitalakshmi S

St John's Medical College

Therapeutic approach for Patients with Nephrotic syndrome (NS) targets pathogenesis caused by deranged immunology . A potential role of B cells has emerged due to the therapeutic efficacy of the B-cell depleting anti-CD20 antibodies (rituximab and ofatumumab) in inducing and/or maintaining a prolonged remission in children with NS Aims To evaluate the role of B cell depletion (due to Injection Rituximab) in patients with nephrotic syndrome

Materials and methods Retrospective analysis (2016 to 2022) of patients with NS treated with Injection Rituximab for reducing the pathogeneic effect of B cells was done. Patients with irregular monitoring were excluded. Using Lyse- wash protocol,subsets of gated Lymphocytes was done using; CD 19 (PE Cy7) , CD20 (V450) for B cells, CD7 (PerCp Cy 5) or CD 3 (APC-H7) for T cells. Equipment used : BD FACS Canto ;Diva software.

Results: 39 patients were evaluated. Of these, 33 pediatric patients, (Male: female was 21:12) , average age of patients was 6.5 years. Clinical diagnosis was mainly Steroid dependent NS (n= 28 cases) . Steroid resistant NS in 3 cases , Lupus nephritis - 1 and CNI toxicity -1 case. 23 cases underwent biopsies, 8 diagnosed as Minimal change disease(MCD), 7 FSGS and 8 showed Mesangial Hyperplasia. Pre and post treatment, the mean B cell% was 19.2 and 0.32 respectively. Average interval between Pre and post treatment testing was 1.35 months . With the drastic fall in B cells, the degree of proteinuria or hypoproteinemia showed improvement. Among 6 adults , Average pre and post treatment B cell% was 14.94 and 1.1 . However there was persistence in proteinuria.

MRD No	AGE	GENDER	CD	BX	BASELINE	T1	R1	Pre ort	post ort
4144085	4	F	SRNS	MCD	16.1	1	0.1	3	0
3147996	7	M	SDNS	MCD	23.1	0.5	0.1	0	0
3147997	7	M	SDNS	MCD	36.3	1	0	0	0
3555003	13	F	SDNS	FSGS	18.4	0.5	0	3	0
3555004	13	F	SDNS	FSGS	21.1	0.2	0.1	3	0
3099918	4	M	SDNS	FSGS	37.07	2	0.35	Tr	0
3099917	4	M	SDNS	FSGS	22.25	2	0.17	Tr	0
3099919	4	M	SDNS	FSGS	14.2	0.2	0.4	Tr	0
3445911	2	M	SDNS	MCD	21	1	0	1	0
2212585	8	F	CNI Tx	MHC	14.3	0.2	0	2	0
3417662	5	F	SDNS		24.7	0.2	0	3	0
3417663	5	F	SDNS		23.7	0.2	0.2	3	0
3449686	2	M	SDNS		8.4	1	0	2	0
3444030	3	M	SDNS	MHC	17.6	1	0.1	4	0
2396208	5	M	SDNS	MHC	18.4	3	0.09	3	0
2396209	5	M	SDNS	MHC	23.8	6	3.53	2	0
2396201	5	M	SDNS	MHC	22.36	1	1.1	4	0
2396203	5	M	SDNS	MHC	22.3	1	0	2	0
2396205	5	M	SDNS	MHC	14.6	1.5	0.4	2	1
2396207	5	M	SDNS	MHC	11.4	1	0.1	3	0
3673310	2	F	SDNS	MCD	12.9	0.2	0	0	0
3734579	5	M	SDNS	FSGS	19.8	0.5	0.4	0	0
4247974	9	F	SDNS		7.1	4	0	0	0
4334573	12	M	SDNS		14.9	4	0	1	0
4247975	9	F	SDNS		8.7	2	0.5	3	0
4436429	2	M	SDNS		20.9	3	0	2	0
4333170	14	F	SLE		35.6	1	1.9	1	0
3245670	6	M	SDNS	FSGS	13.4	0.7	0.3	4	tr
4311190	11	M	SDNS		12.6	1	0.1	2	0
4452541	8	M	SRNS	MCD	19.4	0.5	0.1	3	2
4277103	2	M	SRNS	MCD	13.2	2	0.1	4	2
4109672	4	F	SDNS		27.6	1	0.1	1	tr
3411283	8	F	SRNS	MCD	20.6	0.3	0.6	3	0
avg	6.152	12 f	28sd	8mcd	19.3266667	1.354545	0.3285		
max	14	21 m	3 sr	7 fs	37.07	6	3.53		
min	2	33	1 sle	8mh	7.1	0.2	0		
			1 tox	23 tot					
				10 blank					

Conclusion Injection Rituximab plays a key adjuvant role in the treatment of nephrotic syndrome. For pediatric patients CD19 monitoring was useful in evaluating the effectiveness of treatment.

The Cytometry Society-India
15th Annual Conference & Workshops
26th -29th October, 2023
Theme: From Basics to Multi-Omics

CP13 -Exploring the Clinical, Hematological, and Immunophenotypic Profile of Waldenstrom's Macroglobulinemia: Insights from a Case Series in Eastern India.

Meera.M, Asish Rath, Sushant Vinarkar ,Mayur Parihar1, Sambunath Banerjee , Subhojit Brahma , Munmun Banerjee, Saurav Chowdhury ,Jeevan kumar, Reena Nair , Arijit Nag , Debaranjini Chattopadhyay, Dibakkar Podder, Deepak Kumar Mishra

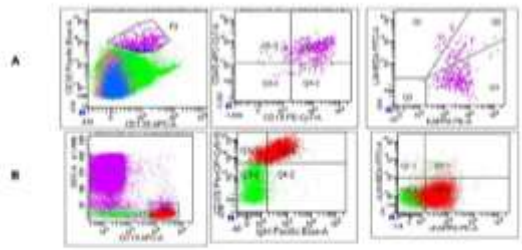
Department of Lab hematology, Tata Medical Centre, Kolkata

Introduction:-Waldenstrom's macroglobulinemia (WM),indolent B-cell lymphoproliferative disorder primarily affecting the bone marrow and associated with IgM monoclonal gammopathy. WM poses diagnostic challenges due to its rarity and heterogeneity.

Aim:- This study aims to characterize the clinical, hematological, and immunophenotypic features of 28 WM cases.

Materials and methods: The confirmed WM cases, established through molecular studies for the MYD88 L265P mutation and biochemical investigations (2012-2023)at our institution were included. All the cases had undergone bone marrow assessments, biochemical studies, flow cytometric analysis(FCM)and molecular studies. We employed an 8-color 5-tube B-Chronic Lymphoproliferative disorder panel, with an additional 11-color plasma cell tube utilized in most cases to characterize the immunophenotypic landscape.

Results:-Our results indicated a group primarily consisting of males, with a mean age of 64 years. The most frequently reported complaint was fatigue (74%). Physical examinations revealed lymphadenopathy in 14.2% and hepatosplenomegaly in 28.2% of cases. Peripheral blood analysis showed rouleaux formation (60%), cold agglutination (3.5%), cryoglobulins (7.1%), and circulating lymphoplasmacytic cells(100%). Anemia was consistently observed. Significant M bands , elevated IgM levels with a predominant IgM kappa pattern in immuno fixation electrophoresis were observed in all cases. Bone marrow examinations revealed atypical lymphoid cell infiltration, plasmacytoid lymphocytes, and plasma cells. FCM- exhibited predominant expansion of clonal B-lymphocytes, displaying vivid expression patterns. Kappa light chain restriction emerged as the most frequent clonality marker (52%) among lymphocytes. Although CD5 and CD10 expression were absent in all cases, dim CD23 expression was discerned in 41.1% of patients. Dim to moderate levels of CD200 expression were apparent in 47% of cases. A notable patients (58%) showed sIgM expression Additional analysis with a plasma cell tube indicated a clonal plasma cell population predominantly revealing kappa light chain restriction(72%) and expressing CD45, CD19, CD27, and CD81. The MYD88 L265P mutation was detected in every case examined. In addition, two cases showed CXCR4 gene mutation as well.



A: Plasma cell tube reveal clonal plasma cells(Kappa restricted) and are positive for CD45 and CD19.
B: B-CLPD tube showing clonal B-lymphocytes(Kappa restricted) expressing CD20 and sIgM.

Conclusion:- WM consistently exhibits unique bone marrow characteristics and a distinctive immunophenotypic profile of abnormal lymphocytes and plasma cells. The identification of clonal lymphocytes and plasma cells in addition to molecular studies can help in achieving a more precise diagnosis as opposed to other B-lymphoproliferative disorders .

CP14 - Paediatric B-ALL with t(1;19): Prevalence, Immunophenotype with Karyotype correlation and outcome from a tertiary care centre

Anab Sayyada, Udayakumar DS, Dhwanee Thakkar, Akshay Gore, Rishab Pandey, Ritu Chadha, Bhawna Jha, Shalini Goel, Neha Rastogi, Satya Prakash Yadav, Renu Saxena.

Medanta-The Medicity hospital

Background: B-cell acute lymphoblastic leukemia (B-ALL) with t(1;19)(q23;p13)/TCF3::PBX1 accounts for 3–5% of B-ALL and shows a characteristic immunophenotype and has good outcomes from intensive chemotherapy. Cases of hyperdiploidy with t(1;19) with different breakpoints has been described. We aimed to determine the prevalence, correlation of immunophenotype with karyotype and outcome of t(1;19) B-ALL treated with the modified Berlin-Frankfurt-Münster (BFM) protocol.

Methods: 136 consecutive paediatric B-ALL patients from January 2018 to August 2023 were screened by cytogenetic analysis and/or reverse-transcriptase PCR. Risk stratification and treatment were done according to modified BFM-95 protocol. Clinical characteristics, flowcytometric immunophenotype and treatment outcomes were analysed.

Results: B-ALL with t(1;19) was detected in 6/136 (4.4%) patients with mean age of 8.5 years (range 3 to 15 years) and equal sex predilection comprising five newly diagnosed B-ALL and one patient presenting with relapse after receiving UK ALL based treatment from outside facility and was lost to follow up subsequently. None of the patients had CNS or testicular disease at presentation. Classical t(1;19)(q23;p13)/TCF3::PBX1 fusion was seen in five cases (3.67%) and all showed a characteristic bright expression of CD19/CD24/CD9/CD10, dim CD20 and CD34 negative immunophenotype. In contrast, the case with hyperdiploidy and t(1;19)(q11;p13) showed immunophenotype of CD19/CD10/CD20/CD34/CD24/CD9/CD13+33. Of the five newly diagnosed B-ALLs, one case with TCF3::PBX1 fusion was classified as high risk due to day 8 poor prednisone response. Four out of five cases have completed intensive phase treatment, were MRD negative on day 33 and day 79 and are in remission with a median follow-up of 21 months (range 17 to 33 months). The high-risk B-ALL patient is currently on intensive phase treatment with MRD negative on Day 33 and Day 79 and on follow-up.

Conclusion: TCF3::PBX1 B-ALL constituted 3.67% of paediatric B-ALLs in our cohort, and showed a predictable characteristic immunophenotype compared with the cases of t(1;19) with hyperdiploidy showing different breakpoints. These patients show good response to treatment on intensive chemotherapy protocol.

CP15 - Adenocarcinoma presenting as intusseption

Dr Shrutika Chopra, Dr Karamjeet singh gill, Dr Manisha Sharma, Dr Neha Sharma, Dr Harjot kaur

SGRD, Amritsar

Adult with bowel interception caused by malignant tumours are fairly uncommon A case of 57 year old female whose intussusception is secondary to moderately differentiated. Adeno carcinoma presented with pain abdomen since five months associated with nausea and vomiting. On Radiology, multiple polyps were identified in ascending colon. Along with mild thickening of approximate 7 to 6 MM. Patient was admitted on histopathology after surgery. It got Case of adeno carcinoma poorly differentiated, infiltrating. Muscularis propria and Reaching up to the serosa. The patient was afterwards discharged without any complication.

CP16 - 11-color single tube high-sensitivity B-ALL MRD: Data from a Cancer care centre in Eastern India

Munmun Banerjee1, Subhajit Brahma1, Sambhunath Banerjee1, Meera M.1, Anirban Kundu1, Pratyush Mishra1, Reena Nair2, Arijit Nag2, Jeevan Kumar2, Vaskar Saha3, Niharendu Ghara3, Sushant S. Vinarkar1, Mayur Parihar1, Asish Rath1, Deepak Kumar Mishra1

1. Deapartment of Laboratory Haematology, Tata Medical Center, Kolkata;
2. Department of Clinical Hematology, Tata Medical Center, Kolkata;
3. Department of Pediatric Oncology, Tata medical Center, Kolkata

Introduction: Minimal/measurable residual disease (MRD) in B-cell lymphoblastic leukemia (B-ALL) is the single most important predictor of treatment outcome. Though achievement of MRD negativity by conventional flow cytometry (MFC) is associated with favorable outcomes, relapse rates in MRD negative patients are 25% or higher in reports. These relapses are possibly driven by low level MRD below the detection level of conventional MFC. Thus, we evaluated a highly sensitive MFC-MRD to detect low level MRD in both pediatric and adult B-ALLs.

Patients and methods: The study included all consecutive patients referred for B-ALL MRD from July-2022 to August-2023. 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 events in all cases. Only the cases where a minimum of 4 million viable events could be acquired were included for final analysis. All samples were acquired on BD FACSLyric 3-laser 12-color (BD Biosciences, San Jose) and analyzed using Kaluza v2.2 software (Beckman Coulter Life science, California).

Results: 321 samples at different time point (post-induction- 133; 41.4%, post-consolidation-91; 28.3%, other time-points-97; 30.3%) were included in the study. Total MRD detected was 31.8% (MRD range 0.0005%-17%, median MRD 0.02%). Post-induction (PI) MRD detected was 39.1% (pediatrics-40.8%; 38/93, Adults-35%; 14/40). Of post-consolidation (PC) samples (n=91) only 10 samples (11%) were MRD detected. Rest of the samples from other time-points showed MRD detectability of 41.2% (40/97). Loss of CD81 was the most informative in our study helping in MRD identification in 81.5% cases followed by CD38 under expression (80.5%) and over expression of CD10 (71.8%) and CD73 (51.4%). CD58 (17.4%) and CD45 (25.2%) were the least informative markers.

Conclusion: High-sensitive MFC-MRD can detect MRD at a much lower level and prevent false-negative MRD rates. High-sensitive MFC-MRD analysis can possibly guide more reliable therapeutic interventions.

CP17 -Expression pattern of new markers in Acute myeloid leukemia and their utility for Measurable residual disease assessment

Dr. Mona Singh, Prof Tembhare, Prashant, Dr. Chaturvedi, Anumeha, Ms Dalvi, Kajal, Ms Sriram, Harshini, Ms Patil, Jagruti, Ms Girase, Karishma, Mr Deshpande, Nilesh, Mr Ghoghale, Sitaram, Dr. Rajpal, Sweta, Dr. Chatterjee, Gaurav, Prof. Patkar, Nikhil, Prof. Subramanian, P.G., Prof. Gujral, Sumeet

TATA MEMORIAL CENTRE, ACTREC

INTRODUCTION: Measurable residual disease (MRD) is the most relevant biomarker for prognosis and treatment efficacy monitoring in patients with AML. Multicolor flow cytometry (MFC)-based MRD monitoring is a widely used and sensitive technique. However, its application is still limited due to the overlap of routinely used antigen expression with normal myeloid progenitors (NMP). Therefore, additional markers are required to increase the applicability and accuracy of MFC- MRD. We studied expression of CD52, CD54, CD97, CD93, CD9 and CD44 in AML and their practical utility in MRD monitoring.

METHOD: 13-16 color MFC-MRD was performed using bulk-lysis and stain method using Cytoflex (Beckman Coulter) and LSR-Fortessa (BD). Data was analyzed using Kaluza-software. Normalized mean fluorescence intensity (nMFI) was calculated for expression intensity. Expression pattern were compared with NMP.

RESULT: A mixed cohort of 60 adult and pediatric patients was studied. Median age of patients was 18 years (range 5-67 years). Of 60, MRD was available in 32 patients and 21/32 were MRD positive. Amongst the 6 markers studied, the highest frequency of LAIP was observed with CD54 in 35/60(58.3%) followed by CD93 in 15/60(25%), CD52 in 12/60(20%), CD97 in 12/60(20%), CD9 in 11/60(18.3%) and CD44 in 11/60(18.3%). The proportion of samples with immune shift in order of "Stable", "Loss" and "Gain" of expression for these markers was as follow: CD54-53.8%, 46.2%, 33.3%; CD93-zero%,100%; CD52-82.4%, 17.6%, zero%; CD97- 40%, 60%, 6.2%; CD9- 20%, 80%, 6.2%; and CD44 40%, 60%, 12.4%.

CONCLUSION: Of the 6 markers studied, CD54, and CD52 showed the highest frequency of LAIP and the best post-treatment stability in their expression. Thus, incorporating CD54 and CD52 in MFC-MRD analysis in AML patients shall further enhance the accuracy and applicability of MRD assay.

CP18 - "Unravelling the Cellular Dynamics of Endometriosis: Immunophenotyping Endometrial-Derived Cells Across Disease Severity"

Safia Begum1, Roya Rozati2, Ayyapathi Mehdi Gautam3 Vikram Aiman Ayyapathi4 Aleem Ahmed Khan1*

1. Centre for Liver Research and Diagnostics, Deccan College of Medical Sciences, Kanchan Bagh, Hyderabad-500058, Telangana, India.
2. Maternal Health Research Trust, Department of Obstetrics and Gynecology, Banjara Hills, Hyderabad-500034, Telangana, India

Introduction: Endometriosis, a common gynaecological disorder, involves the ectopic presence of endometrial-like tissue, causing chronic pelvic pain and infertility. Despite its prevalence, the disease's origins remain poorly understood, warranting a thorough exploration of cellular dynamics. At the cellular level, endometriosis exhibits dynamic changes, influencing its development and progression. These alterations encompass shifts in cell behaviour, signalling pathways, and interactions with the microenvironment, shaping the disease's pathophysiology. To decode the mechanisms underlying disease severity, our study delves into immunophenotyping endometrial-derived cells across mild to severe endometriosis, focusing on CD105, CD90, and CD73 markers, shedding light on vital cellular mechanisms.

Materials and methods: Biopsies from clinically diagnosed endometriosis patients (n=25) and healthy controls endometrial tissues (n=25) were collected during laparoscopy and processed in phosphate buffer saline (PBS). Single cells were isolated via collagenase digestion, then cultured in DMEM/F12 with 10% FCS for 5 days. Enriched cells were subsequently stained with CD73, CD90, and CD105 for flow cytometric analysis to assess mesenchymal transition percentages and relative gene expression analysis of CD73, CD90, and CD105 was quantified using SYBR-Green.

Results and Discussion: Flow cytometry analysis revealed an increased percentage of cells expressing mesenchymal markers (CD-90, CD-73, and CD 105) in severe cases of endometriosis. Relative gene expression analysis showed significantly higher fold values for (CD-90, CD-73, and CD 105). This suggests a potential role of epithelial-to-mesenchymal transition (EMT) in endometriosis progression and invasiveness.

Conclusion: This study reveals insights into the immunophenotyping of endometrial cells in endometriosis, emphasizing CD105, CD90, and CD73 as potential progression biomarkers. Exploring EMT's role may guide targeted therapies. Further validation and functional research are needed.

CP19 - BPDCN: Clinical and Immunophenotypic spectrum

Mohammed Aakif K A, Merlin Priyanka, Phaneendra Datari, Kotteeswari Kathirvel, Madhavi Maddali, Poonkuzhali Balasubramanian, Fouzia NA, Sushil Selvarajan, Uday Kulkarni, Anu Korula, Arun Kumar Arunachalam, Biju George, Aby Abraham, Vikram Mathews

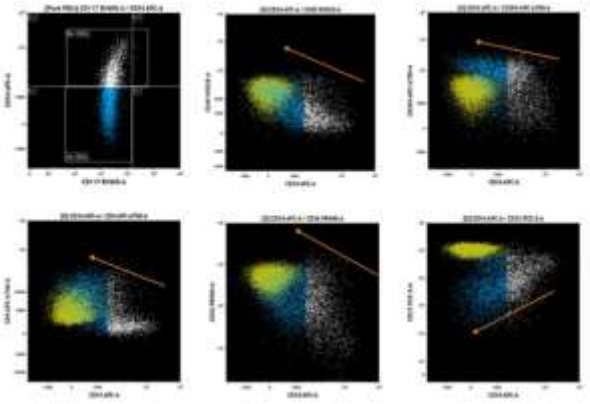
Haematology laboratory, B-Block B-707, 7th Floor, Christian Medical College-Ranipet Campus, Ratnagiri, Kilminnal-632517.

Introduction: Blastic Plasmacytoid Dendritic Cell neoplasm (BPDCN) is a rare and aggressive neoplasm that arises from precursors of plasmacytoid dendritic cells (PDC). Although this entity has been known for decades, it often causes diagnostic conundrums in day-to-day practice.

Aim: In this study we aimed at establishing the clinical and immunophenotypic spectrum of BPDCN and its differences from AML with monocytic differentiation and mature PDC neoplasms.

Materials & Methods: BPDCN cases diagnosed at the department of Haematology, CMC-Vellore between 2012 and 2023 were analyzed. Immunophenotype was analyzed on bone marrow samples at presentation that were processed by lyse-stain method, acquired using BD FACSCaliburTM, BD FACSCanto IITM, BD FACSDivaTM and BD FACSLyricTM cytometers and analyzed using BD FACSSuiteTM. Patients' clinical details were taken from electronic patient records.

Results: During the study period, 8 cases of BPDCN were diagnosed. Median age at presentation was 56 years and all the cases were males. 3/8 cases had hepatosplenomegaly, 4/8 cases had lymphadenopathy and 2/8 cases had ulcerative skin lesions. Characteristic immunophenotype was bright expression of CD4, CD56 and CD123 in the absence of lineage defining markers like cMPO, CD19, cCD3, CD64 along with absence of CD34 and dim to negative CD117. CD304 was used in 3/8 cases of which only 1 was positive. Absence of CD64, CD11b and CD14 helped to distinguish BPDCN from cases of AML with monocytic differentiation among the 8 cases of the latter that were compared. Presence of CD56 helped to distinguish from mature plasmacytoid dendritic cells in 2 cases of the latter that were compared. Variations in expressions of CD304, CD33, CD117 and CD36 could be explained by the stage of maturation of PDCs at which the neoplasm arises.



Conclusion: Study of maturation pattern of PDCs gives us an insight into possible variations in marker expression among BPDCN cases. Immunophenotype can differentiate BPDCN from AML with monocytic differentiation and mature PDC neoplasms with confidence.

CP20 -COMPARING EOSINO-5 MALEIMIDE (EMA) AND HAEMATLOGICAL PARAMETERS IN DIAGNOSIS OF HEREDITARY SPHEROCYTOSIS

Dr.Ruchi Agrawal , Dr. Harsukh Toprani , Dr.Bankim Patel Dr.Tushar Toprani

Toprani Advanced Lab System, Vadodara

ABSTARCT: Eosino-5 maleimide (EMA) is considered the gold standard for diagnosis of HS. However, routinely acquired haematological parameters like percentage of microcytes, percentage of hypochromic cells, reticulocyte counts, and percentage of immature reticulocytes on Sysmex XN1000 have also been used as a low-cost alternative as suggested by Mullier et al. In this study, we characterise the cases where there was discordance between the 2 techniques.

METHOD: The EMA study was done on 70 requested sample for HS by using beckman coulter DX flex 5 colour flowcytometer and results were campared with hematological parameters, on Sysmex instrument XN 1000 by running the sample in CBC plus retic mode. The levels of Hb, Reticulocytes (Ret), Immature Reticulocytes Fraction (IRF), Hypochromic Erythrocytes (Hypo-He) and Microcytic Erythrocytes (MicroR) were determined on EDTA samples. Retics/IRF ratio and MicroR and MicroR/Hypo-He ratios were calculated.

Results: HS was characterized #Retics > 80000/µl. Mild HS(Hb > 12g%) have Ret#/ IRF ratio greater than 19. Moderate (Hb 8- 12g%)and severe HS(Hb < 8 g%) have Ret#(/µl)/IRF (%) greater than 7.7 and also have increased MicroR and MicroR/Hypo-He Ratio.The results of HS from hematological parameters has in our study has sensitivity of 96.7% and specificity of 92.3%. There was only 1 case that was diagnosed on EMA but missed on haematological parameters,was 9 month old baby. Similarly, there were 3 cases that were positive by haematological parameters but had negative EMA.

CONCLUSION: Hematological parameters are simple and fast diagnostic method, and can be used as an excellent screening tool for HS. In this study, we characterised the discordance between the 2 techniques.
KEYWORDS Hereditary spherocytosis , Eosino-5 maleimide, Haematological parameters

CP21 –Role of Flow cytometric immunophenotyping in lymphoma diagnosis:A tertiary care centre experience

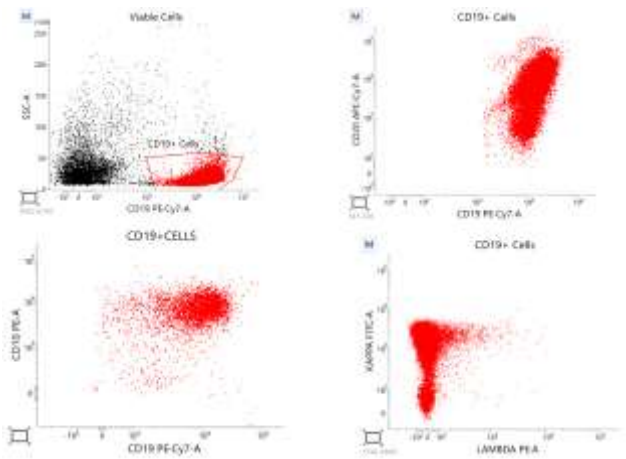
Dr Vidisha Mahajan, Dr Khushbu Kasundra, Dr Shanaz Khodaiji

PD Hinduja Hospital and Medical Research Centre

Introduction: Chronic lymphoproliferative disorders (CLPD) are a heterogenous group of lymphoid malignancies derived from T, B and NK cells in mature lymphocyte. WHO classification of lymphoid neoplasm is based on morphological and immunophenotypical characteristics and in some cases, molecular markers.We have looked into profile of suspected CLPD cases received for immunophenotyping at our institution over last 2 years.

Aim and Objective: To study profile of lymphoproliferative disorders (LPDs) diagnosed by flow cytometry at a tertiary health care centre. Method: The list of samples subjected to flow cytometry in view of suspicion of LPD, over a period of two years from 2021 to 2023, was obtained and the details of the patients retrieved from the medical records. Morphology of their lymphoid cells in peripheral blood and bone marrow aspirates and immunophenotype as inferred from flow cytometry were carefully studied.

Result: A total of 110 cases of were evaluated, which had a median age of 65 years and male to female ratio of 1.75:1. Of these 110 cases, 103 cases were of B-CLPD and 7 cases were of T-CLPD. Chronic lymphocytic leukemia (CLL) was the most common form, constituting 49.5% (51 cases) of all. Other B-CLPD identified in our study included 4 cases of burkitt lymphoma, 4 cases each of monoclonal b cell lymphocytosis and marginal zone lymphoma, 3 cases each of follicular lymphoma (FL) and mantle cell lymphoma (MCL) and 2 cases of diffuse large B cell lymphoma (DLBCL). Two unusual cases were also diagnosed including a double hit lymphoma presenting as a plasmablastic lymphoma at one site and follicular lymphoma at a different site. A case of T prolymphocytic lymphoma was also seen.



Conclusion: Flow cytometry is an essential tool for diagnosis of chronic lymphoproliferative disorders. A basic minimal panel is required for diagnosis of lymphoproliferative disorders. A comprehensive set of additional antibodies is required to diagnose the rare lymphomas involving the bone marrow especially when lymph nodes are inaccessible.

CP22 - Genetic Heterogeneity in CD10 -ve B-ALL and its effect on outcomes

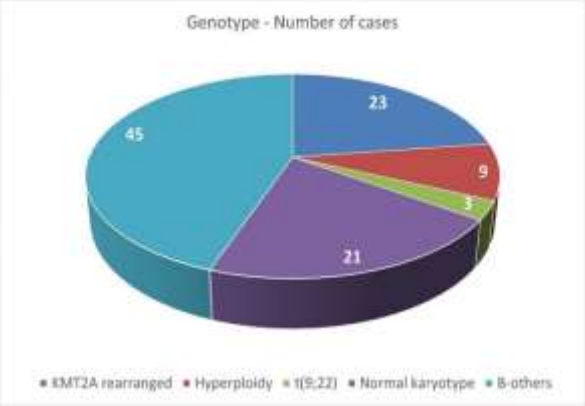
Kathirvel Kotteeswari Phaneendra Datari, Gayathri Kuppusamy, Madhavi Maddali, Poonkuzhali Balasubramanian, Sushil Selvarajan, Uday Kulkarni, Anu Korula, Arun Kumar Arunachalam, Biju George, Aby Abraham, Vikram Mathews
Haematology laboratory, B-Block B-707, 7th Floor, Christian Medical College-Ranipet Campus, Ratnagiri, Kilminnal-632517.

Introduction: B-ALL is a genetically heterogenous neoplasm with well-established cytogenetic and molecular subtypes that influence outcomes. Certain immunophenotypic aberrancies are associated with cytogenetic or molecular subtypes and guide the workup of patients accordingly. Here we discuss the genetic heterogeneity among B-ALLs with aberrant CD10 loss, which is known to be associated with KMT2A re-arrangement.

Aim: To look for genetic heterogeneity among CD10 -ve B-ALLs and evaluate its effect on outcomes.

Methods: The study included all the CD10 -ve B-ALLs diagnosed at our department between 2012 and August 2023. Cytogenetic and clinical details were retrieved from electronic patient records. Follow up residual disease assessments were included wherever available.

Results: A total of 101 cases of CD10 -ve B-ALL were analyzed. KMT2A rearrangement was seen in 23/101 (22%) cases. Hyperploidies were seen in 9/101 cases, t(9;22) in 3/101 cases and B-others were 45/101. Normal karyotype with no clonal abnormalities were seen in 21/101 (20%) cases. Among the 101 cases, 39 had follow up with residual disease assessments (MRD). MRD was positive in 19/39 cases (~50%). Among the MRD positive cases, 4/19 were KMT2A rearranged, one case had t(9;22), 3 cases had hyperploidy – including triploid and tetraploid cases. Normal karyotype cases showed bulk residual disease positivity in 5 cases.



Conclusion: KMT2A rearrangement was seen in 22% of CD10 -ve B-ALLs in our cohort. Irrespective of the genetic alteration, CD10 -ve B-ALLs showed bulk MRD positivity at end induction, suggesting poorer outcomes.

The Cytometry Society-India
15th Annual Conference & Workshops
26th -29th October, 2023
Theme: From Basics to Multi-Omics

CP23 - Identification and targeting of key genes in whole transcriptome profiling of an ovarian cancer patient post hysterectomy with bilateral salpingo-oophorectomy

Dr Muqtadir Baig Mirza1, Dr Roya Rozati*2, Dr Fareeduddin Quadri Syed1, Dr Vikram Aiman Ayapati3, Dr Gautam Mehdi Ayapati3, Syeda Hajera Begum1, Shaik Iqbal Ahmed1, Dr Aleem Ahmed Khan1
CMH Research and Innovation, Bolarum, Secunderabad, Telangana, India

Abstract: Ovarian cancer is the fifth leading cause of death among women. In 2021, nearly 21,000 women were diagnosed and approximately 14,000 succumbed to ovarian cancer in the United States alone. Poor diagnosis and disease re-occurrence are major problems and almost half of the cases already reached III to IV stages when diagnosed. There is a lack of comprehensive investigation of disease initiation and progression, including gene expression changes during early metastatic colonization, which further leads to poor prognosis and treatment.

Methodology: RNA Sequencing of ovarian cancer patient from liquid biopsy (blood) was done and matched with serious ovarian carcinoma to evaluate the changes in gene expression and ovarian cancer (OC) progression to identify and target genes effectively. Up-regulated differentially expressed genes (Up-DEGs) were identified by analyzing the gene expression levels. Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed. This approach shows vital changes in gene expression.

Results: The gene data significantly showed up-regulation of 3179 differentially expressed genes out of which 112 genes were related to cancer progression and metastasis. JAK-STAT & Notch pathways were identified to be key pathways for disease progression. PRKCB, IL6 and CSF1R are highly expressed gene which could be the key genes that can be targeted by Midostaurin, Siltuximab and Dovitinib respectively. Collectively, our study provides a novel and comprehensive insight into the gene expression changes of the non-invasive peripheral liquid biopsy of OC. The expression heterogeneity–based predictive model may help guide prognostic management and precision targets for OC patients.

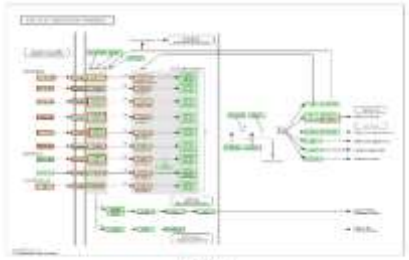


Figure 1

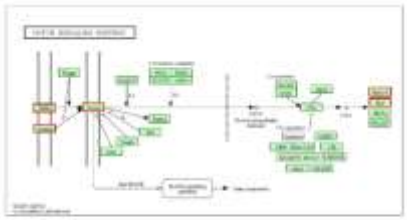


Figure 2

Figure 3

CP24 - Resolving the Diagnostic conundrum of small lymphocyte-like plasma cell leukemia (SLL-PCL) through peripheral blood multi-parametric flow-cytometry (MFC)

Avinash Gupta* , Neha Singh, Prateek Das, Rohitkumar Kori, Nilesh Dhole, Sujit Kumar, Anil Singh, Malini Garg
HBCH/MPMMCC, TMC, Varanasi

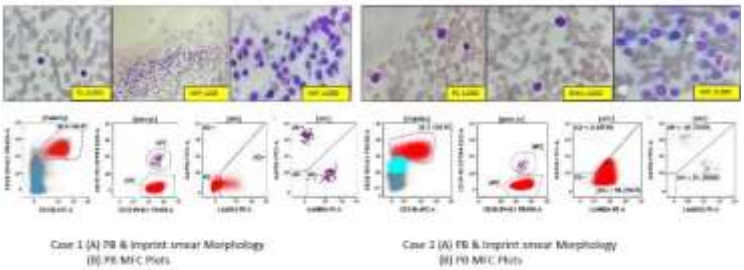
Introduction: Primary plasma cell leukemia (PCL) diagnosis requires ≥5% of circulating plasma cells in peripheral blood (PB) smears. In rare scenarios plasma cells with atypical morphology (small lymphocyte-like), can be a potential mimicker of a mature B-cell neoplasm. Here we are presenting two such cases where PB MFC played a pivotal role in accurately diagnosing these cases as Plasma cell leukemia with small lymphocyte-like morphology (SLL-PCL).

Clinical Information : Case 1. A 48-year-old male presented with lower back pain and had anaemia (hemoglobin 7.3 g/dL), thrombocytopenia (platelet 93 x 109/L), osteolytic lesions, IgG lambda monoclonal protein, elevated serum creatinine (2.41 mg/dL) and serum calcium was 10.5mg/dL.

Case 2. A 50-year-old female presented with lower back pain radiating to right lower limb and had anaemia (haemoglobin, 8.1 g/L), osteolytic lesions, IgG lambda monoclonal protein and elevated serum beta-2 microglobulin (11.4 mg/L). PB smear and bone marrow aspirate in both the cases revealed small, atypical lymphocyte-like cells which lead to a morphological diagnosis of lymphoproliferative disorder. In addition to bone marrow aspirate MFC, a 10 color MFC assay was also performed on PB which revealed 11% and 17.0% abnormal clonal plasma cells, respectively with similar immunophenotype as seen in the bone marrow. Hence a diagnosis of Small lymphocyte-like Plasma cell leukemia (SLL-PCL) was made in both the cases. (Image Case 1 & 2)

Discussion: Plasma cells can display a diverse range of morphological characteristics. In the cases described above, the presence of small lymphocyte-like morphology in PB created a diagnostic conundrum. MFC not only resolved this morphological ambiguity but also established the clonality of the circulating plasma cells.

Conclusion: These clinical cases illustrate the diagnostic challenges posed by the rare presentation of PCL with atypical morphology (SLL-PCL). The diagnostic process integrated clinical assessments and data from various ancillary investigations, underscoring the pivotal role of PB MFC in achieving a definitive diagnosis of SLL-PCL.

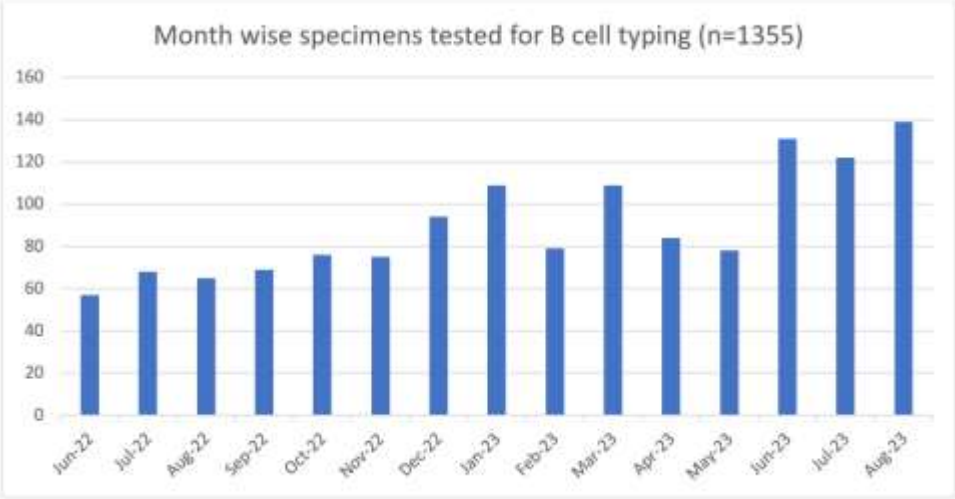


CP25 - B cell immunophenotyping in patients with neurological autoimmune disorders at a tertiary neurocare centre

Manjunatha M V, Pavithra M, Lakshmi P, Ruthu N, Seena V, Nalini A, Netravathi M

National Institute of Mental Health and Neurosciences (NIMHANS)

The incidence of auto immune neurological disorders such as multiple sclerosis, Neuromyelitis Optica, Myasthenia gravis etc have been on the rise in recent years. Consequently, the neurology clinics at NIMHANS have been diagnosing and treating a high number of patients with such disorders. Depletion of B cells with anti-CD20 monoclonal antibodies such as Rituximab is a commonly used approach to treat these patients. Monitoring of B cell frequencies in peripheral blood by Flow Cytometry has become an essential component in clinical management of neurology patients who are on immunosuppressive therapy. The clinicians need to know the baseline levels of CD19 and CD20 positive B cells in peripheral blood before starting Rituximab therapy. Further, the B cell levels are monitored during follow-up visits to the neurology clinic at 3 to 6-month intervals so that re-population of the B cells is detected in a timely manner to consider a repeat dose of the B cell depleting antibody. As most patients who seek clinical care at NIMHANS hospital are Below Poverty Line, the Flow Cytometry Laboratory at Department of Neurovirology, NIMHANS initiated the clinical flow cytometry service to support improved and economical care for these patients from June 2022. The B cell typing assay is based on anti-CD45, anti-C19 and anti-CD20 fluorescent antibody staining of whole blood specimens. A total of 1355 specimens were received for the assay from June 2022 to August 2023. The details of month wise specimens received from patients with demyelinating disorders, neuromuscular disorder etc will be presented in addition to clinical diagnosis, age distribution, analysis of repeat specimens received from individual patients etc.

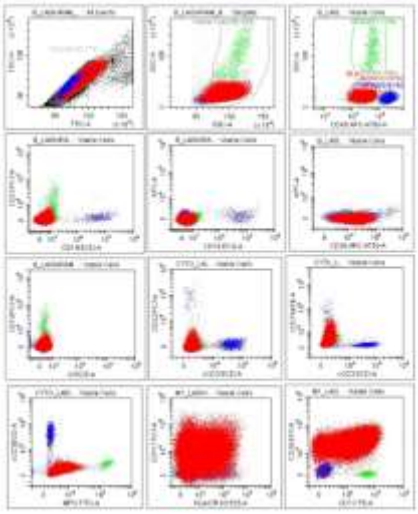


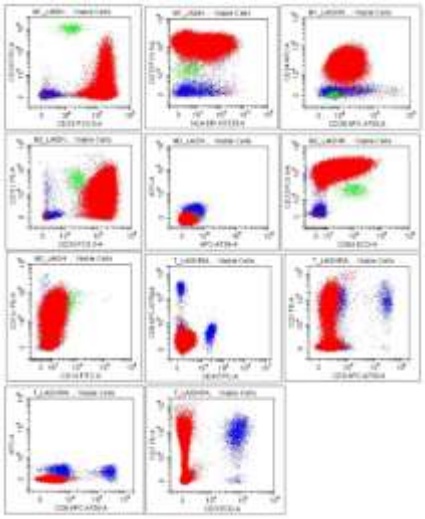
CP26 - Case of T-LBL relapsing as Acute myeloid leukaemia

RK Banashree Devi and Dr Irom Anil Singh

Jawaharlal Institute of Medical Sciences, Imphal, Manipur

A 25 year old male patient presented with generalised lymphadenopathy and swelling of the left arm in the month of September 2021. Biopsy from the Left supraclavicular lymph node was performed. On microscopy, architecture was effaced with sheets of medium to large sized tumour cells. On immunohistochemical staining, these cells are positive for CD3, Tdt, CD2, CD4, CD8 and CD7. Ki67 was positive on 90% cells. These cells were negative for CD20, CD30, CD117 and CD138. The case was diagnosed as T cell lymphoblastic lymphoma. Patient was started treatment according to BFM-90 protocol. In October 2021, patient developed left sided pleural effusion. Minimal residual disease analysis showed 9.2% (end of phase 1), 0.47%(end of phase 2) and 0.22% (maintenance phase). Possibility of allogenic bone marrow transplant was suggested however patient could not afford. Palliative therapy with 6MP/Methotrexate was continued. On 4th December 2022 Patient was discharged with a suggestion to consult local oncologist or hematologist. In the month of April 2023 (26/4/2023), Patient presented with Haemoglobin of 6.3gm/dl, TLC of 18800/cu mm with 90% blasts. Flowcytometric immunophenotyping from the peripheral blood showed 94% CD45dim, low SSC blasts with heterogenous positivity for CD 117, CD38, CD13, CD64 CD11c, HLADR and CD7. These blasts were homogenously positive for CD34 and CD33. MPO was positive. These blasts were negative for CD4, CD8, CD3, cCD3, CD14, CD15, CD22, CD19, CD10 and cyCD79a. A final diagnosis of Acute myeloid leukaemia was made. Patient was treated with Decitabine and HiDAC. Complete haemogram examination on 18.5,2023 showed haemoglobin of 5.7gm/dl, TLC of 310/cumm, platelet count of 30,000/cu mm and no blasts.





CP27 - COMPARING CONCORDANCE BETWEEN BD FACSLyricTM AND SYSMEX XF-1600TM INSTRUMENTS FOR EXPRESSION OF ACUTE LEUKEMIA SCREENING ANTIGENS

Dr. Aditi Mittal, Dr. Anil Handoo, Pradeep K. Rai, Dr. Tina Dadu

BLK-Max Superspeciality Hospital

Background: Flow cytometry (FCM) is a preferred technique for immunophenotyping of cells and widely used for characterization of blast cells for diagnosis and classification in acute leukemia (AL) cases in accordance with other clinical findings. The current study compared sensitivity and performance of two different flow cytometers XF-1600TM manufactured by Sysmex Corporation, Japan and BD FACSLyricTM from BD Biosciences by using 8C acute leukemia screening panels from BD Biosciences and its impact in diagnosis.

Methods: A total of 20 peripheral blood (PB) or bone marrow (BM) samples of suspected acute leukemia subjects have been examined for validated 8-color-AL antigen screening panel by utilizing reagents and staining protocol from BD Biosciences. To evaluate the instrument performance, same stained samples were acquired on two different flow cytometers Sysmex XF-1600TM considered as "BD-XF" group and BD FACSLyricTM as "BD-Lyric". Data analysis was performed by using VenturiOneTM analysis software marketed by Sysmex Corporation. The populations of interest (White blood cells and blast cells) were gated based on their SSC and CD45 pattern. The gated BLAST cells were displayed on the following plots: SSC vs CD34, CD34 vs CD19, cCD3 vs CD7, sCD3 vs CD34, CD79a vs MPO. BLAST cells were analyzed for percentage population and stain index for AL screening antigens. The results from both flow cytometers were compared for instrument performance.

Results: There was no significant difference observed between BD-XF and BD-Lyric groups. Blast cells showed the equivalent percentage and stain index for acute leukemia screening antigens. This study showed that both instruments are equivalent for sensitivity and performance for acute leukemia screening antigens on blast cells. These results signify that with a given set of reagents, performance of both flow cytometers Sysmex XF-1600TM and BD-FACSLyricTM are comparable for sensitivity and resolution of screening antigens for AL diagnosis.

Conclusions: This case study highlights the comparable performance of Sysmex XF-1600TM with BD FACSLyricTM which ensures precise detection of BLAST cells immunophenotype. The Immunophenotyping results suggest that both the Sysmex XF-1600TM and BD-FACSLyricTM were comparable with regards to the percentage population and stain index, hence newly launched XF-1600 can offer an efficient FCM tool in hematological malignancies

CP28 - COMPARATIVE STUDY OF A COMPLETE SOLUTION OF REAGENTS AND INSTRUMENT FROM SYSMEX CORPORATION AND BD BIOSCIENCES FOR CHARACTERIZATION OF BLAST CELLS BY FLOW CYTOMETRY METHOD IN ACUTE LEUKEMIA SUBJECTS

Dr.Aditi Mittal, Dr.Anil Handoo, Pradeep K.Rai, Dr.Tina Dadu

BLK-Max Superspeciality Hospital

Background: Hematolymphoid malignancies (HMs) are the most common form of uncontrolled growth of hematopoietic and lymphoid tissues. In current study, we compared sensitivity and performance of Fluorochrome (FL) conjugated antibodies and related reagents from both manufacturers on the respective flow cytometers XF-1600TM (Sysmex-XF) and FACSLyricTM (BD-Lyric) by using 8 color acute leukemia screening panels and its impression in diagnosis.

Methods: A total of 20, peripheral blood (PB) and bone marrow (BM) available combinations of samples having suspected acute leukemia human subjects were stained in two sets for validated 8-color-AL antigen screening panel by utilizing reagents from Sysmex and BD Biosciences. One set of samples were stained by utilizing Sysmex reagents and acquired on Sysmex XF-1600TM flow cytometer (Sysmex-XF) and second set of samples were stained by utilizing reagents from BD Biosciences and acquired on BD FACSLyricTM (BD-Lyric) flow cytometer. Data analysis was performed by using VenturiOneTM analysis software. The populations of interest (White blood cells and blast cells) were gated based on their SSC and CD45 pattern.

Results: Blast cells were analyzed for percentage positive population and stain index of MPO, CD79a, cyCD3, CD7, sCD3, CD10, CD19 and CD34 antigens expression. Unsurprisingly, no considerable difference was observed in percentage population of blast cells expression for all antigens except CD34. Interestingly, better and significant ($P=0.04$) difference was observed in percentage population of CD34 expressing blast cells in Sysmex-XF group. On further investigation, Sysmex-XF group showed a considerably ($P<0.01$) better stain index for CD34 antigen.

Conclusions: This case study highlights that Sysmex reagents are comparable with BD Biosciences reagents for sensitivity and resolution, while the difference observed for CD34 is a further matter of investigation. Overall, these results indicate that the immunophenotyping results produced by both the Sysmex-XF and BD-Lyric group were comparable with regards to the antigen expression pattern and stain index.

CP29 - Comprehensive analysis of Euroflow B-CLPD panel in a rare case of Hairy cell leukemia-variant.

Dr Pooja Sharma, Dr Namrata Punit Awasthi, Dr Yatendra Parashar

Department of Pathology[1], Department of Clinical Hematology[2], Dr Ram Manohar Lohia Institute of Medical Sciences

Introduction: Hairy cell leukemia-variant (HCL-v) is a distinct clinico-biological entity, exhibiting differentiable clinical and hematological profile, vis-à-vis Classical Hairy cell leukemia (HCL). The neoplastic lymphoid cells differ also in terms of cytomorphology, immunophenotype and response to conventional HCL therapy. This propelled the need for change in terminology from HCL-v to SBLPN in fifth edition of WHO classification.

Case summary: 75-year-old man presented with complaints of generalized body weakness and abdominal distension. There were no complaints of fever, night sweats or weight loss. Spleen was palpable 4 cm below costal margin with no significant peripheral lymphadenopathy or hepatomegaly. Initial investigations showed mild leucocytosis (11,200/µl), thrombocytopenia (1.08 X 10^3/ µl), and mild anemia (hemoglobin 11.5 gm/dl). Differential counts revealed lymphocytosis (63%), cells were small to medium in size, displayed clumped chromatin, prominent single nucleolus, moderate cytoplasm, and circumferential hairy projections. Differential diagnoses considered were Splenic marginal zone lymphoma (SMZL), HCL-v, Splenic diffuse red pulp small B-cell lymphoma (SDRPL) and HCL.Flowcytometric immunophenotyping revealed a monoclonal B cell population ($CD19^+$ with bright expression of surface Kappa light chain). These cells co-expressed bright CD20, CD22, CD11c and CD305 and were negative for CD200, CD103, CD25, CD5, CD10 and CD23.

Discussion: HCL, SMZL, SDRPL and HCL-v are all characterized by involvement of peripheral blood, bone marrow and spleen; and have overlapping immunophenotype. Flowcytometry contributes to differential diagnosis, essential for purpose of management and follow-up. HCl-v cells are consistently negative for CD25 and occasionally positive for CD103, whereas HCL cells are invariably positive for CD25 and CD103. Contrarily, CD103 is negative and CD25 can either be positive or negative in SMZL. SDRPL are negative for CD25 and CD103.

Conclusion: HCL-v is a clinically more aggressive mature B-cell lymphoma. The management depends on early detection of HCL-v through careful history, physical examination, morphology, and flow cytometry.

Key words: Hairy cell leukemia-variant, Flowcytometry, B-CLPD

CP30 - CD371 expression in B-lymphoblastic leukemia and its correlation with recurrent genetic abnormalities

Sneha Ann Oommen, Simpy Raj, Anumeha Chaturvedi, Anu Singh, Ghogale S, Deshpande N, Badrinath Y, Girase K, Rajpal S,Chatterjee G,Patkar N, Gujral S, PG Subramanian, Tembhare P
ACTREC, TMH

Introduction: CD371 expression in B-lymphoblastic leukemia and its correlation with recurrent genetic abnormalities Introduction: CD371(CLL-1) is a transmembrane glycoprotein usually expressed on normal myeloid cells and most of the myeloid blasts. Aberrant expression of CD371 was observed in DUX4-rearranged B cell precursor ALL (BCP ALL). However, extensive data of CD371 expression in B-ALL and its expression pattern in patients with disease-defining recurrent genetic abnormalities is limited. With the advent of anti-CD371-immunotherapy, we aimed to assess the expression of CD371 in patients of all B-ALL and study its correlation with underlying genetic abnormalities.

Methods: Expression of CD371 (fluorochrome-PE, clone- 50C1) was evaluated in consecutive B-ALL patients from January 2020 to December 2021 using 12-color MFC immunophenotyping on DxFLEX flow-cytometer. Data was analysed using Kaluza-softwareV2.1. CD371-expression was correlated with demographic details, cytogenetics, and molecular genetics data including DUX4 rearrangement.

Results:CD371 was assessed in 690 B-ALL patients (412 paediatric and 278 adult-B-ALL). CD371 was positive in 64 patients (9.28%) of B-ALL. Incidence of CD371 expressing B-ALL was higher in paediatric patients (59.3%, n-38). RNA-sequencing data was available in 484/690 patients and DUX4 rearrangement was detected in 14/484 (2.9%) patients. CD371 was positive in 12 of these 14 patients (85.7%, p-value <0.0001). CD371 was also detected in patients with other genetic abnormalities including 3/20 (15%) B-ALL with KMT2A-rearrangement (p-value 0.63), 7/99 (7.1%) B-ALL with BCR::ABL1 (p-value 0.61) and 1/180 (0.5%) patients of B-ALL with hyperdiploidy. Co-expression of CD371 and CD2 was noted in 5/14 patients (35%) with DUX4-rearrangement (p-value <0.0001).

Conclusions: Aberrant expression of CD371 was significantly associated with DUX4 rearranged B-ALL. However, it was also positive in other genetic abnormalities including KMT2A rearrangement and BCR::ABL1. This highlights the need for extensive study of CD371 expression in B-ALL with recurrent genetic abnormalities and underlines the utility of anti-CD371 immunotherapy in such patients.

CP31 - Expression pattern of ROR-1 in B cells and its clinical utility in Measurable Residual Disease (MRD) monitoring

Dhanashree Patil, Steffi Varghese, Priyanka Dhende Harshini Sriram,Sitaram Ghogale, Nilesh Deshpande, Karishma Girase, Jagruti Patil, Gaurav Chatterjee, Nikhil Patkar, Sumeet Gujral,P. G. Subramanian,Sweta Rajpal, Prashant Tembhare
TATA MEMORIAL CENTRE ACTREC

Introduction: Multiparametric flow cytometry (MFC) is a popular technique for MRD analysis. However, its applicability is still challenging in proportion of cases that show drug-induced antigen modulation or in which early (Day <28) BM MRD was performed as these cases show restricted maturation patterns. Hence to improve the sensitivity and specificity of MFC-based MRD monitoring we studied the expression of new marker ROR-1 in B-cells including leukemic blasts of B-cell lymphoblastic leukemia (B-ALL) patients and evaluated its practical utility in MFC-based MRD analysis.

Methods: Expression-patterns of ROR-1 was studied in leukemic-blasts from 102 B-ALL patients (age group 0-62 years), 15 staging BM and 25 MRD samples using 13-colour panel, acquired on DxFlex (Beckman Coulter) and data was analyzed using Kaluza-v2.1-software. ROR1 expression intensity and pattern were studied with percentage, mean fluorescent intensity (MFI) and coefficient of variation (CV).

Results: The ROR-1 was highly expressed in stage2 (CD10+CD19+CD34-) hematogones with median MFI(range) and CV(range) of 4.06(2.32-8.04) and 37.51(32.89-52.35) respectively. The median MFI(range) of ROR1 expression in stage1 (CD10+CD19+CD34+) hematogones, mature B cells and leukemic blasts were 3.035(2.24-7.79), 3.825(0-5.89) and 2.90(-0.73- 16.36) respectively. Out of 102 B-ALL cases ROR-1 was positive in 4 cases. 23.6% of 102 B-ALL showed CD19+CD10+CD34- immunophenotype. Among 25 MRDs, 15 cases were MRD positive of which 1 case showed heterogeneous ROR- 1 expression.

Conclusion: ROR1 is expressed in stage-II hematogones. It is distinctly useful especially to distinguish CD19+CD10+CD34- leukemic blasts from stage-II hematogones, thus reducing the false positive MRD rates in the detection of high-sensitive MFC-based MRD assay.

CP32 - Minimal Residual Disease analysis by Single Tube Ten Color Flow Cytometry in B Cell Acute Lymphoblastic Leukemia: A Single Centre Experience.

Kusum Gupta, Prakhar Gupta, Manoj Sarkar, Shobha, Ruchi Gupta, Manish Kumar Singh, Dinesh Chandra, Sanjeev, Rajesh Kashyap, Khaliqur Rahman

Dept of Hematology, SGPGIMS, Lucknow

Introduction: Detection of MRD by flow cytometric or molecular techniques has been recognized as one of the most important measures of treatment response and predictor of relapse. It is now routinely evaluated in all treatment protocols. In comparison with molecular techniques, the flow cytometric detection of MRD has the advantage of general applicability, high speed and lower cost, and hence has been the preferred method used for MRD detection by many laboratories. In B cell precursor MRD evaluation, it is important to distinguish the small residual disease from normal B cell precursors. We present our experience with single tube 10 color BCP MRD evaluation in the patients being treated with BFM 95 protocol.

Materials & Methods: Retrospective analysis of all consecutive BCP ALL MRD analysis done over a period of 36 months (NOV 2019 – NOV2022). • Sample: EDTA Anti-coagulated Bone marrow aspirate samples. Samples were collected at different time point • T1: Post Induction • T2: Post Consolidation • T3: Any other time point • Cell preparation: First pull sample-Bulk Lysis. • ANTIBODIES Used are CD45/CD38/CD34/CD19/CD10/CD20/CD73/CD86/CD123/ CD304. • A minimum of 20 clustered events with a minimum of 2 leukaemia associated immunophneotype (LAIP) was considered as positive for MRD. **RESULTS:** 812 BM-samples were analyzed from 312 patients during 36 month period (NOV 2019 – NOV2022). • T1= 296 samples, T2=280 samples, T3=236samples • MRD was detected in 69(23.31%) samples at T1, 27(9.64%) at T2 & 17(7.20%) At T3 time period. MRD proportion ranging from 0.002 to 9.6%, Median-0.25%

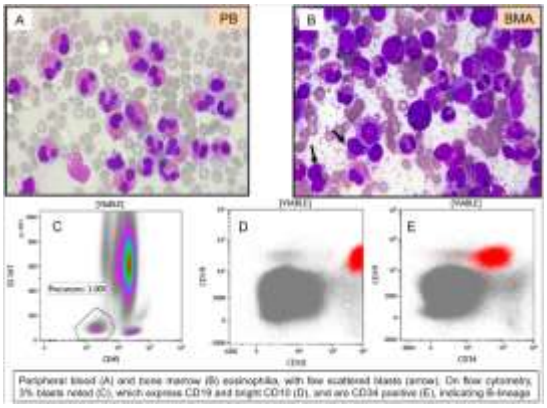
Conclusion: Single tube 10 color analysis is a reliable and sensitive way of B ALL MRD detection • First marrow pull sample - increases the cell yield in MRD evaluation • Increasing the events analyzed, has higher propensity to pick up lower MRD values • The age did not have any effect on the incidence of MRD.Post Induction MRD ∝ with the disease relapse.

CP33 - What's hiding behind the eosinophils? – Flow cytometry unravels the mystery in an interesting case of pediatric hypereosinophilia

Atul Kumar Jain, Nabhajit Mallik, Praveen Sharma, Sreejesh Sreedharanunni, Man Updesh Singh Sachdeva, Amita Trehan1
Postgraduate Institute of Medical Education & Research, Chandigarh

INTRODUCTION: Eosinophilia in children is usually caused by non-neoplastic conditions such as parasitic infections, allergies and drug reactions. Clonal eosinophilia is usually associated with rearrangement of certain genes like PDGFRA, PDGFRB and FGFR1. We present a case of severe hypereosinophilia, where the bone marrow had a surprise in store for us.

CASE REPORT: An 8-year-old boy presented with fever, cough and right shoulder pain for 1 week. CBC revealed mild anemia (Hb- 10.3 gm/dl) and thrombocytopenia (platelets- 117 x109/L), with marked leucocytosis (TLC-225.7 x109/L). PBF showed 93% eosinophils, with an absolute eosinophil count of 209.9 x109/L. With such profound eosinophilia, bone marrow examination was performed to rule out a clonal cause. Bone marrow also showed eosinophilia with 68% eosinophils and precursors, while there was reduction in the erythroid and granulocytic series cells. Upon closer examination, some interspersed immature cells were also noted (~15%). Flow cytometry was performed, which revealed 3% abnormal B-lineage blasts in the marrow. Fluorescent in-situ hybridization did not reveal any gene rearrangement associated with clonal eosinophilia. However, a signal pattern consistent with 14q32 (IGH) rearrangement was seen in 4% of CD19 sorted cells. This, however, was below recommended cut-off for positivity. Considering morphological and immunophenotypic features, possibility of B-acute lymphoblastic leukemia with IGH::IL3 fusion [t(5;14)] was suggested. The patient was started on ALL-therapy, and his eosinophilia gradually recovered. At day 35, the patient was doing well, his TLC was 7.4 x109/L with <1% eosinophils, bone marrow did not reveal excess of blasts or eosinophils, and no measurable residual disease was detected by flow cytometry.



CONCLUSION: B-lineage acute lymphoblastic leukemia can rarely present with marked eosinophilia, with low blast percentage in blood/ bone marrow. A high index of suspicion is required, and flow cytometry can be of immense help in reaching a diagnosis.

CP34 - T-cell prolymphocytic leukemia – A report of two cases

Dr. Rishab Gupta , Dr. Sindhura Lakshmi K.L., Dr. Ananth Pai
Kasturba Medical College, Manipal

INTRODUCTION

T-cell prolymphocytic leukaemia is a proliferation of prolymphocytes, which are post-thymic mature T-cells. Incidence is about 2% of mature lymphoid leukaemias in adults >30 years.

CASE REPORTS

Case 1 - 62-year-old male presented with generalised weakness and bilateral cervical, epitrochlear, and axillary lymphadenopathy.
Case 2 - 65-year-old male presented with cellulitis and increased TLC on routine evaluation. Peripheral blood leucocytosis was noted with >90% abnormal small lymphoid cells in both the cases. Case 2 showed classical morphology while Case 1 showed small-cell morphology. Peripheral blood immunophenotyping of both showed CD2, CD3, CD4, CD5, and CD7 positivity and loss of CD1a. Case 1 was CD4+/CD8+, while Case 2 showed CD4+/CD8-. Immunohistochemical analysis of Case 1 showed a loss of CD34 and Tdt. Case 1 was started on 2 cycles of vincristine, daunorubicin, l-asparaginase and 8 cycles of bendamustine. Total counts normalised in seven months. Patient presented with 60,000 TLC one year later following which he was lost to follow up. Case 2 was treated outside but presented 5 months later with pleural fluid involvement.

DISCUSSION

Morphologically, 75% T-PLLs show small/medium-sized cells with non-granular basophilic cytoplasm with blebs, round to irregular nuclei with prominent nucleoli. A Small cell variant (25%) exhibits scant cytoplasm, small nuclei containing condensed chromatin and inconspicuous nucleoli. T-prolymphocytes are characteristically positive for CD2, CD3, CD5, and CD7, and negative for TdT and CD1a. Co-expression of CD4/CD8 is a distinct feature of T-PLL and rarely seen in other post-thymic T-cell neoplasms. Mature T-cell neoplasms with leukemic presentation such as ATLL, SS and T-LGLL can mimic T-PLL. Other differentials include T or B ALL, CLL/SLL, and mantle cell lymphoma.

CONCLUSION

Case 2 showed classical morphology while Case 1 showed small-cell morphology. Immunophenotypic features of both cases pointed towards T-PLL. Both cases showed an aggressive and unpredictable clinical outcome

CP35 - To study the correlation of new markers with MRD positive BALL cases by multiparametric flowcytometry.

Arpita Joshi, Sabina Langer, Amrita Saraf, Pallavi Prakhar, Nitin Gupta, Manas Kalra, Jyoti Kotwal

Sir Ganga Ram Hospital, New Delhi.

Introduction: Minimal residual disease by multiparametric flowcytometry is an integral part of BALL treatment and follow up. We recently standardised BALL MRD on 12 colour flowcytometer. To decide which is the most promising MRD marker we analysed on MRD data of past 3 years. Material and

Methods: This is a retrospective study of a 3 year time period, from Sept 2021 to Sept 2023. In our study , all BALL cases whose post induction and post consolidation MRD was done at our department were analysed. Further study was done in the 23 cases that were MRD positive to compare the significance of markers used in our panel and the ability of the markers to diagnose MRD positivity. This was done after standardisation and validation of our panel after calculation of LOD and LOB.

Results: Out of 23 positive MRD cases 13 (57%) were males and 10 (43%) were females. All cases ranges from 1 year to 70 years of age. Markers underexpressed in leukemic blasts were CD38 (21/23), CD45(13/23), CD81(16/23) as compared to hematogones. Markers overexpressed in leukemic blasts were CD58(16/23), CD73(13/23), CD123(10/23), CD19(2/23), CD10(13/23).

Conclusion: It is a constant effort to discover the new MRD markers in order to make the test more rapid and cost effective. From a practical point of view, the most optimal MRD detection panel is the one that detects and quantify the MRD in a time and resource constraint manner.

CP36 - Acute megakaryoblastic leukemia in pediatrics age group

Dr Shivani goyal, Dr.R M Jaiswal, Dr.Shweta Bansal, Dr.Shweta Sharma, Dr.Priyanka Sharma, Dr.Manju Raghva

Mahatma Gandhi medical College

BACKGROUND: Acute megakaryoblastic leukemia (AMKL) is a rare malignancy affecting megakaryocytes, platelet-producing cells that reside in the bone marrow accounting for 3–10% of primary AML in childhood. Median age of presentation in children is 6 years (ranging from 3 months to 16 years).

CASE HISTORY:
Patient is 2 years old male came with complaints of intermittent fever & loss of appetite, history of epistaxis since 20-25 days . On examination patient having B/L cervical, axillary lymphadenopathy & hepatomegaly.

RESULT:
CBC showed Hb – 6.7g/dl , platelet count-27× 10^3/µl, TLC-27.1×10^3/µl. 21% blasts were seen on PBF .Bone marrow aspiration & biopsy picture was consistent with Acute leukemia (38% blasts). Immunophenotyping findings favour Acute Megakaryoblastic Leukemia - moderate positive for Cytoplasmic CD41&CD61 , D36, CD33, CD4, dim positive for CD117 and CD 45 ,negative for CD20, CD16, cCD3, CD5 ,CD7 , CD2, MPO, CD3, HLADR and CD79a. Immunohistochemistry showed CD61-Focal dim positive and MPO negative in blast cells. Cytogenetic analysis showed translocation t (1, 22) which favors the diagnosis of Acute megakaryoblastic leukemia.

CONCLUSION:
Acute megakaryoblastic leukemia is a rare malignancy in pediatric age group .Its prompt diagnosis was facilitated by integration of morphology, flow cytometry and advanced cytogenetics under one roof .This helped in immediate management of patient in a right direction.

The Cytometry Society-India
15th Annual Conference & Workshops
26th -29th October, 2023
Theme: From Basics to Multi-Omics

CP37 - Utility of CD48 in detection of residual T-lymphoblasts in flow cytometry-based MRD assay

Tharageswari S, Parveen Bose, Arun Kumar, Nabhajit Mallik*, Praveen Sharma, Sreejesh Sreedharanunni, Man Updesh Singh Sachdeva, Pankaj Malhotra(a), Amita Trehan(b)

Department of Hematology, Clinical Hematology and Medical Oncologya and Pediatrics, Post Graduate Institute of Medical Education and Research, Chandigarh, India, 160012.

Introduction: Measurable residual disease (MRD) detection in T-lineage acute lymphoblastic leukemia (T-ALL) is crucial, but may be difficult at times, especially using 8/10 color panels. The present study aims to evaluate the role of CD48 in distinguishing T-blasts from mature T and NK cells, and to explore its role in MRD detection.

Methods: All cases of T-ALL (diagnostic and MRD) reported in the Department of Hematology, PGIMER from April 2022 to August 2023 were analysed for CD48 expression in T blasts, mature T cells, CD4/CD8 dual negative (presumably gamma-delta) T cells, NK cells and mature B-cells. CD48 expression was calculated as median fluorescence intensity (MFI), and the MFI of the different populations were normalized by expressing it as a ratio of the population of interest to that of the MFI of mature B-cells.

Results: Fifty-one T-ALL cases were diagnosed, with median age of presentation being 13 (range 2-63) years, and a male:female ratio of 4.7:1. A total of 79 flow cytometry (FC) data files of MRD-assay were analysed, of which 26 were MRD positive. The median normalised CD48 MFI of T lymphoblasts(analysed from 51 diagnostic and 26 MRD-positive cases) was 0.2, while it was 1.4 in mature T cells(51 diagnostic and 26 MRD cases), 1.09 in gamma-delta T cells(41 MRD cases) and 1.06 in NK cells(41 MRD cases). The difference in nMFI of T lymphoblasts compared to the other three populations was statically significant (p value < 0.0001). The nMFI in diagnostic B-ALL and AML blasts were also calculated in 15 cases each, and were found to be 0.19 and 0.28 respectively.

Conclusion: Normalized CD48 MFI was significantly lower in T-blasts compared to mature T cells, gamma-delta T cells and NK cells. CD48 can be a useful and dependable addition to the existing FC panel for detecting MRD in T-ALL.

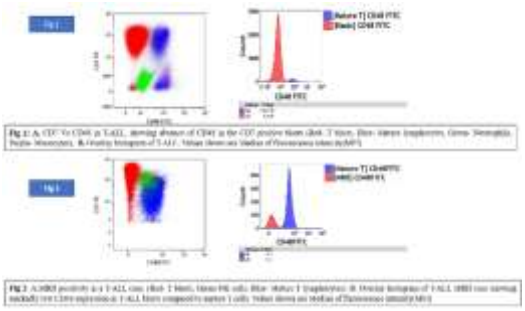


Table 1

	T Blasts	Mature B cells	Mature T cells	NK cells	CD4 negative CD8 negative T cells(presumably Gamma delta T cells)
Median CD48 MFI	0.6	4.4	6.4	3.1	3.5
Median Normalised CD48 MFI (nMFI)	0.2	-	1.4	1.06	1.09
Comparison of CD48 nMFI with that of CD48 nMFI of T blasts	-	-	P<0.0001	P<0.0001	P<0.0001

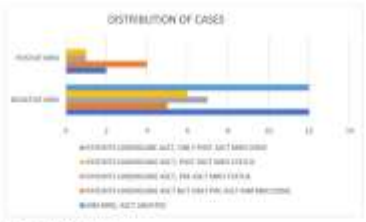
CP38 - MINIMAL RESIDUAL DISEASE ASSESMENT IN MULTIPLE MYELOMA USING FLOW CYTOMETRY : AN EXPERIENCE AT TERTIARY CARE CENTRE

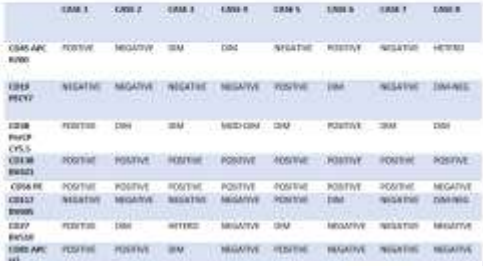
DR BHUMIKA GUPTA, DR KHALIQUR RAHMAN, DR RUCHI GUPTA, DR DINESH CHANDRA, DR MANISH KUMAR SINGH, DR SANJEEV YADAV, DR RAJESH KASHYAP

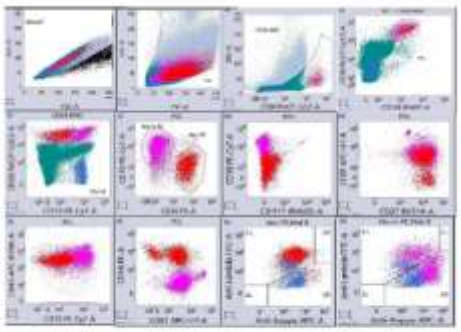
Sanjay Gandhi Post Graduate Institute Of Medical Sciences, Lucknow, Uttar Pradesh

Introduction: Minimal residual disease evaluation (MRD) by flow cytometry (FCM) in multiple myeloma (MM) is one of the most commonly used method to assess the overall response and progression free survival.

Material/method: FCM MRD analysis was performed on diagnosed and treated patient for Multiple myeloma, who had achieved very good partial response and were planned/undergone autologous bone marrow transplant (ASCT). 50 first pull bone aspirate MRD samples of 35 patients were processed using Bulk-lyse-stain method and analyzed on a single tube 10 color MM MRD panel with a target of acquiring 2.5 million events in each case.







Result: Majority of the patients were males (60%) with median age of 47 years ±10.02. Only 8 MRD bone marrow samples had detectable residual disease with male preponderance (p =0.375). 21/35 patients underwent ASCT. 7 patients had pre and post ASCT day 100 MM-MRD assessment, where one patient was pre ASCT MRD positive, followed by negative Pre and post ASCT day 100 MRD. One patient was negative pre ASCT but relapsed post ASCT. Other 7 had post day 100/200 MRD assessment, rest 7 patients had only pre ASCT MRD evaluation but post ASCT day 100 MRD was awaited. 14/35 patients were planned for ASCT. CD56 was the most consistent positive marker in detecting the abnormal clonal plasma cells along with downregulation of CD19, CD45, CD38, CD27 and CD81. CD117 was not upregulated which is usually observed in abnormal plasma cells.

Conclusion: This study has helped us analyze several treatment milestones such as end of induction therapy, early or late stages of maintenance or day 100/200 post ASCT. Flow cytometric MRD detection can be used a potential biomarker to evaluate the efficacy of various treatment strategies and provide better judgment for overall survival in multiple myeloma patients.

CP39 - Blastoid variant of Mantle cell lymphoma- a diagnostic enigma

Dr.T. Rama Devi, Co-Authors: 2)Dr Prerna Walia 3) Dr.Tara Nair,4)Dr Jyoti Sawhney, 5)Birva
GCRI ,ASARVA

Introduction:
Blastoid variant of Mantle cell lymphoma (MCL) is considered as an aggressive mature B-cell neoplasm characterized by t(11;14) translocation with overexpression of cyclin D1 and has poor prognosis. Blastoid variant can occur with conventional MCL (cMCL) and Leukemic non-nodal MCL (nnMCL), which have differing biology and clinical behaviour. It is essential to exclude CLL , splenic marginal zone lymphoma, DLBCL,Lymphoblastic lymphoma and Burkitt Lymphoma.

Aim :
To present clinical and hematological profile and right diagnostic approach of rare cases of blastoid variant of Mantle cell lymphoma

Material and Methods:
Electronically retrieved reports of CBC, peripheral smear, Bone marrow aspirate, Lymph node and bone marrow biops Immunohistochemistry(IHC) done on Ventana autostainer ,flow cytometry in peripheral blood sample on BD FACS Canto II with Euroflow consortium panel for acute leukemia and CLPD panel were utilized in these cases. Cytogenetics for t(11,14)by FISH was done in one case .

Results:
3 cases presented acutely with peripheral smear suggestive of blasts or atypical lymphoid cells. Age ranged from 41-79 years , Hb range (3.9 – 8.8 g/dl) , Total leucocyte count range (2.3- 868x10 9 /L),Platelet (18-88x109 /L) ,LDH range(637 —7417 IU/L). Lymph nodes were significantly enlarged in one case where the blastoid morphology was secondary to nodal MCL while in the other two cases had de novo blastoid presentation of MCL.

Conclusions:
Blastoid variant of MCL is a high risk MCL with an aggressive clinical course. Flow cytometry and IHC helps in early diagnosis and treatment initiation. Two cases received Bendamustine and Rituximab while one case succumbed to his illness before treatment.

The Cytometry Society-India
15th Annual Conference & Workshops
26th -29th October, 2023
Theme: From Basics to Multi-Omics

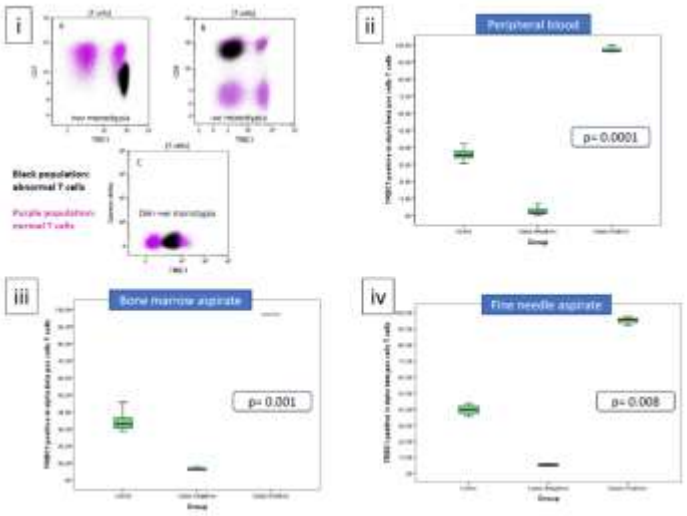
CP40 - Role of TRBC1 as a single-antibody flow cytometric marker for T-cell clonality detection in mature T cell neoplasms

Yukim Dong, Nabhajit Mallik, Parveen Bose, Arun Kumar, Kajol, Sreejesh S, Amanjit Bal, Radhika Srinivasan, Gaurav Prakash, Man Updesh Singh Sachdeva.
PGIMER, CHANDIGARH

Objectives: Diagnosing mature T-cell neoplasms (MTCNs) is a challenge, with the major difficulty lying in establishment of clonality. This study aims to evaluate the utility of TRBC1 as a single-antibody flow cytometric (FC) marker for T-cell clonality and compare it with established methods of clonality detection, i.e. TCR gene rearrangement (molecular) and V-beta repertoire analysis (FC).

Methods: 28 MTCN patients were included in the study. A single-tube 10-color flowcytometry panel (including TRBC1 and other T-cell markers) was used for immunophenotyping and clonality detection. TCR gene rearrangement studies and flowcytometric V-beta repertoire analysis were also performed for comparison wherever feasible.

Results: The most frequent MTCN was T-LGL leukemia (42.8%), followed by TFH-Angioimmunoblastic type and T-cell prolymphocytic leukemia (17.8% each), Mycosis fungoides/Sezary syndrome (10%), and one case each of ALCL, Hepatosplenic T cell lymphoma and Systemic EBV positive TCL. The Male:Female ratio was 1:1.3, and median age 49.6 years. Samples used for testing included peripheral blood (50%), bone marrow (42.8%), FNA (17.8%), and pleural fluid (3.5%). The most common immunophenotypic aberrancy was downregulation of CD5 and CD7 (50% each); 16 cases were CD8+, 11 cases CD4+ and 1 case was dual positive. TRBC1 analysis helped detect clonality in 26 out of 28 (92.9%) cases, with 3 distinct patterns: negative monotypia (69.2%cases), positive monotypia and dim positive monotypia (15.4% cases each) (Fig i). Median TRBC1 expression in MTCLs was statistically significant compared to that in T cells from normal samples (PB, bone marrow and FNA; Fig.ii, iii and iv). Compared to established methods of clonality detection, sensitivity and specificity of TRBC1 was 94.7% of 92.9% respectively.



Conclusion: TRBC1 is a simple and accurate alternative to the established methods of T-cell clonality assessment. It's integration into routine diagnostic panels is likely to improve the sensitivity of detecting MTCNs by flow cytometric immunophenotyping

CP41 - Mixed-Phenotype Acute Leukemia (MPAL)- A Case Series Of 3 Rare Cases

Beulah Priscilla Maddirala, Dr Gurleen Oberoi, Dr Beena Chandra Sekhar, Dr Reena Nakra, Dr Vandana Lal

National Reference Lab, Dr Lal Pathlabs, Rohini sector

BACKGROUND: Mixed-phenotype acute leukemias (MPALs) are a heterogeneous group of rare leukemias constituting approximately 2%-5% of all leukemias, in which assigning a single lineage of origin is not possible. They are diagnosed by either the presence of antigens of more than one lineage or by the presence of dual population of blasts belonging to two or more lineages.

AIMS & OBJECTIVES: To present a case series of three rare cases of MPAL with a spectrum of clinical presentation and their immunophenotypic profile.

Material and Methods: We analysed peripheral blood/marrow samples (EDTA/ Heparin) in 3-laser 8 color FACS Canto II BD Biosciences, San Jose, CA. for which daily calibration was performed and tested using 7 color set up CST beads and one flow set up beads. These cases were diagnosed based on immunophenotypic features as per the World Health Organization (WHO) 2016 guidelines.

RESULTS: Case 1: 59/M presented with fatigue and leukocytosis. Flow cytometry done on peripheral blood showed B/Myeloid MPAL, with a complex karyotype and t(9;22)(q34.1;q11.2); BCR-ABL1 positive genotype.
Case -2: 19/M presented with fever and pancytopenia. Flow cytometry done on bone marrow sample showed T/Myeloid MPAL, normal karyotype and negative for mutations in comprehensive molecular panel. Case-3: 7/F child presented with rashes and thrombocytopenia showed B/Myeloid MPAL on immunophenotyping.

CONCLUSION: MPAL is a complex entity with heterogeneous clinical, immunophenotypic, cytogenetic, and molecular features. Multiparametric flowcytometry by using comprehensive antibody panels is a primary tool for diagnosis. Subsequent cytogenetic and molecular analysis for further prognostic stratification and treatment modalities are important.

Keywords: Multiparametric immunophenotype, mixed-phenotype acute leukemias, BCR-ABL1

CP42 – Role of multiparameter flowcytometry in diagnosis of lymphoplasmacytic disorders and correlation with MYD88 mutation.

Surbhi Dahiya, Sabina Langer, Priyanka Moule, Vandana Arya, Nitin Gupta, Jyoti Kotwal

Sir Gangaram Hospital, New Delhi

Introduction: Waldenström macroglobulinemia/lymphoplasmacytic lymphoma (WM/LPL) is a rare, indolent B-cell non-Hodgkin lymphoma. WM is defined by ≥10% bone marrow lymphoplasmacytic cell infiltration regardless of the IgM peak level. The characterization of the lymphoplasmacytic cells by morphological evaluation is difficult and needs a more sensitive method especially to differentiate from Multiple myeloma and other B-NHLs. Here, we present the utility of flowcytometry (FCM) in the evaluation of LPL/WM.

Methods: FCM analysis of both B-cells and plasma cells was performed on a series of 24 clinically suspected cases of WM/LPL using 12-colour, 3 laser flowcytometry done on a BD FACSLyric™ flow cytometer. MYD88 mutation along with other ancillary tests was performed.

Results: Of total 24 cases, 87.5% (21), 8.3% (2) and 4.2% (1) cases were of WM, IgM MGUS and B-NHL, respectively. MYD88 mutation could be performed on six cases of WM, from which 83.3% (5) were positive and 16.6% (1) case was negative. Kappa clonality was detected in 80.5% (19/24) and lambda in 20.8% (5/24) cases. On flowcytometry, we could detect seven more cases of WM whose serum monoclonal band and mutational studies were later available. The monoclonal B-cells were found in 21/21 WM (100%) and 2/2 MGUS (100%) cases. Monoclonal plasma cells were detected in 19/21 WM (90.5%) and 1/2 MGUS (50%). There was a clear difference in the immunophenotype of LPL plasma cells (CD19+, CD20+,CD45+) versus myeloma plasma cells (CD19-, CD20-, CD56+). There was a continuum of expression of CD138 on the clonal B cells and plasma cells which was not seen in control samples.

Conclusion: Flowcytometry in addition to the IgM paraprotein and mutational analysis for WM/LPL offers a promising and rapid technique which can help orient the correct diagnosis. The immunophenotype of plasma cells and the CD138 continuum pattern of clonal B-cells could contribute to place FCM at the forefront of WM diagnosis.

CP43 - A study of immunophenotypic profile and aberrancy pattern of 15 cases of Plasma cell Leukemia- An experience of a stand alone reference Laboratory.

Gurleen Oberoi MD, DM, Beulah Pricilla, MD, Beena Chandrashekhar, PHD, Reena Nakra, MD, Vandana Lal, MD
National Reference Laboratory, Dr. Lal Path Labs. Pvt. Ltd.

Context: Plasma cell leukemia (PCL) is a rare, life threatening and therapeutically challenging plasma cell neoplasm and deserves a prompt identification to start the most effective treatment. Increased use of multi-parameteric flow cytometry and a broadening array of available reagents for surface and cytoplasmic staining of specific antigens have improved our diagnostic and prognostic abilities in comparison to conventional methods.

Objective: To study the spectrum of immunophenotypic profile and aberrancy pattern of circulating neoplastic plasma cells in PCL. The clinical spectrum and other laboratory parameters were also studied (wherever available).

Design: Prospective analysis of patients diagnosed as PCL in a stand-alone reference laboratory.

Patients and Methods: A total of 15 patients with diagnosis of PCL were evaluated. Peripheral blood from these cases were processed by 2 tube 8 color multiparametric flowcytometry. The samples were processed using stain-lyse-wash method. Flowcytometric analysis was done on BD FACS Canto II (Becton Dickinson, San Jose, CA, USA) using BD FACSDiva™ v8.1.3 software.

Results: Plasma cell population was identified by strong CD38 and CD138 expression, in conjunction with the scatter properties. All the cases (100%) showed moderate to bright expression of CD38 & were negative for CD19 . CD138 showed dim to moderate bright expression in 91% of cases. Cytoplasmic kappa and lambda light chain restriction was found in 55% & 27% of cases, respectively and was found to be equivocal in 18% of the cases. The most frequent aberrant markers demonstrated in decreasing order of frequency were CD56 (64%), CD200 (64%), CD117 (18%),and CD20(9%). Myeloid markers, CD13 & CD33, were detected in one case.

Conclusion: The demonstration of phenotypically abnormal neoplastic circulating plasma cells by flowcytometry can be very useful for rapid and reliable detection of plasma cell leukemia, especially to differentiate it from it's mimckers like B cell lymphomas with plasmacytoid morphology

The Cytometry Society-India
15th Annual Conference & Workshops
26th -29th October, 2023
Theme: From Basics to Multi-Omics

CP44 - Diagnostic and Prognostic Utility of Flow Cytometric Enumeration of Aberrant hMICL–Expressing Circulating Leukemic Stem Cells in BCR-ABL1–Negative Myeloproliferative Neoplasms

Gurleen Oberoi MD, DM, Rishi Dhawan MD ,DM, Jasmita Dass MD, DM, Seema Tyagi MD, Ganesh Vishvanathan MD, DM, Mukul Agarwal MD, DM, Tulika Seth MD, Manoranjan Mahapatra MD, Renu Saxena MD

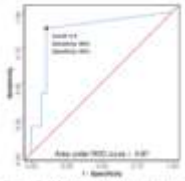
National Reference Laboratory, Dr. Lal Path Labs. Pvt. Ltd.

Context: BCR-ABL1–negative myeloproliferative neoplasms (MPNs)—including primary myelofibrosis (PMF), polycythemia vera (PV), and essential thrombocythemia (ET)—lack specific discriminators and have overlapping features. Subcategorization is challenging, especially distinguishing ET from prefibrotic myelofibrosis (prePMF). hMICL (CD371) is a promising leukemic stem cell (LSC) marker that lacks expression on CD34+CD38– cells in normal and regenerating bone marrow, making it a highly specific marker for LSCs and perhaps a crucial marker for subcategorization of MPNs.

Objectives: To evaluate the utility of flowcytometric enumeration of circulating CD34+CD38– hMICL+ cells for subcategorizing BCR-ABL1–negative MPNs; to correlate this analysis with DIPSS score; and to determine the discriminatory cutoff for this subset that will identify PMF, including prePMF.

Design: In 54 BCR-ABL1–negative MPN patients (PV, 15; ET, 13; overt MF, 13; prefibrotic myelofibrosis, 8; post-PV/ET myelofibrosis, 5) who presented to the hematology department of a tertiary care center in North India over 18 months, bone marrow examination and mutational analysis were done. The following cell subsets were analyzed using peripheral blood flow cytometry: CD34+, CD34+CD38+ (committed progenitor cells), CD34+CD38– (stem cells), and CD34+CD38– hMICL+ (LSCs)

Results: A significant difference in the distribution of the CD34+CD38– hMICL+ subset among the MPN subgroups found: The median in the overt MF, prefibrotic MF, and post-PV/ET MF groups was 2.8% , 4.15%, and 3%, significantly more than in the PV and ET groups (median, 0%; P<0.001). A discriminatory cutoff for the CD34+CD38– hMICL+ subset of 0.9 or greater predicted all myelofibrosis with a sensitivity and specificity of 88% and 89%. This also helped to differentiate prePMF from ET. A significant correlation (ρ=0.41; P=0.036) between the proportion of CD34+CD38– hMICL+ cells and the DIPSS score found. Every 1.72% increase in this subset increased the DIPSS score by 1 unit.



Conclusion: The CD34+CD38– hMICL+ subset appears to be a reliable marker and can be used as a robust diagnostic tool, prognostic marker, and potential target for therapy. Further studies in larger cohorts are needed.

CP45 - Differentiating T/Myeloid MPAL vs ETP-ALL without cMPO and cCD3

Phaneendra Datari, Gayathri K, Kotteeswari Kathirvel, Madhavi Maddali, Poonkuzhali Balasubramanian, Fouzia NA, Sushil Selvarajan, Uday Kulkarni, Anu Korula, Arun Kumar Arunachalam, Biju George, Aby Abraham, Vikram Mathews

Christian Medical College - Vellore

Introduction: T/Myeloid Mixed Phenotypic Acute Leukaemia (T/M-MPAL) and Early Thymocyte Precursor Acute Lymphoblastic Leukaemia (ETP-ALL) are two entities belonging to a spectrum of neoplasms arising from a heterogenous group of Multi Potent Progenitors (MPP) before and just after entering the thymic medulla respectively. Immunophenotypically these entities are differentiated by the co positivity of cCD3 and cMPO for T/M-MPAL versus lone positivity of cCD3 in ETP-ALL.

Aim: Since these entities form a biological continuum between a heterogenous myeloid and lymphoid committed MPPs, we hypothesized that using a ratio of myeloid to lymphoid marker expression excluding cMPO and cCD3, would help in differentiating T/M-MPAL and ETP-ALL

Methods: In the training data set, we included all the T/M-MPAL cases diagnosed at our centre between 2012 and August 2023 and an equal number of ETP-ALL cases. In the validation cohort, we included all the ETP-ALL cases (including the cases used for training) that were diagnosed at our centre between March 2021 and August 2023. Marker expressions were classified into five tier system and scores of 4,3,2,1 and 0 were given for 'Bright', 'Moderate', 'Dim', 'Dim to negative' and 'Negative' respectively. For calculation of ratios, CD13, CD33, CD117 and HLADR were considered as myeloid markers, and CD2, sCD3, CD4, CD5, CD7 and CD8 were considered as T-lymphoid markers. All data was acquired and analyzed on BD FACSLyricTM equipment and BD FACSuiteTM software. Results: For the training cohort, 6 T/M-MPAL cases and 6 ETP-ALL cases were included. A cut-off ratio of 1 (M:T) showed the highest true positive rates and low false positive rates in the training cohort in identifying T/M-MPAL. This cut-off was used on a validation cohort which comprised of 26 cases of ETP-ALL. Cut-off of 1 showed a positive predictive value of 85% in the validation cohort. No single marker expression could differentiate between T/M-MPAL and ETP-ALL. Review of the false positive cases (n=4) showed high side scatter and stronger myeloid marker expression with a dim cCD3 expression that resulted in the diagnosis of ETP-ALL. Of the 4 cases, 2 had follow up at our centre and one case with progressive disease showed downregulation of cCD3 at MRD assessments.

Conclusion: Ratio of myeloid to lymphoid marker expression can be used as a reliable surrogate to differentiate between T/M-MPAL and ETP-ALL in cases of ambiguous cMPO and cCD3 expression.

CP46 - Clinicopathological features of T- Large Granular Lymphocyte Leukemia- A single institute experience

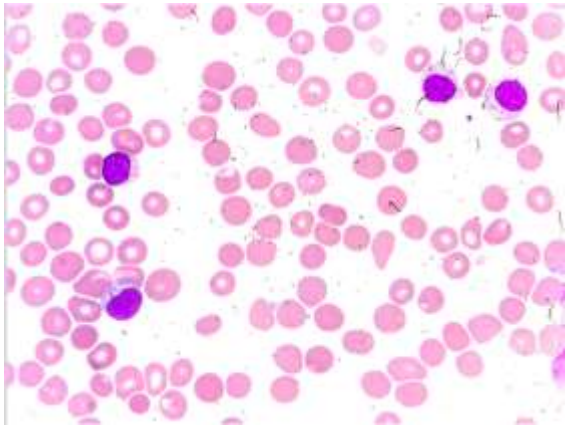
Ipra Singh, Rajesh Kashyap, Khaliqur Rahman, Ruchi Gupta, Dinesh Chandra, Manoj Sarkar

SANJAY GANDHI POST GRADUATE INSTITUTE OF MEDICAL SCIENCES, LUCKNOW

Introduction: T-cell large granular lymphocytic leukemia (T-LGL) is a rare clonal hematological disorder. The reported worldwide incidence is 2–3% of mature small lymphocytic leukemia, while it is unknown in Indian scenario. T-LGL is characterized by persistent increase in LGLs (2 to 20×109 /L) on peripheral blood in absence of a reactive cause. ~40% patients are asymptomatic at presentation and one-third have associated autoimmune disorder. Also, there is infiltration with LGLs in bone marrow and tissues, leading to cytopenia and splenomegaly. The disease is indolent and responds well to treatment.

Material and methods: In this retrospective study from January 2019 to September 2023, all patients diagnosed as CLPD in our department were evaluated. Their medical records were retrieved from computerized Hospital Information System. Those patients who were confirmed to have T-LGL on flow-cytometry were further analysed. The information about clinical presentation, lab investigations and treatment were recorded.

Results: Out of 341 patients suspected as CLPD, flow-cytometric analysis revealed 300(88%) as B-cell neoplasm, 24(7%) as T-cell neoplasm {including 9(2.6%) T-LGL}, 2(0.6%) as NK-cell neoplasm and 15(4.4%) with no clonal population. Mean age of presentation- 57.3 years, male:female ratio=1.25:1. Fatigue and dyspnea were common symptoms, along with fever(2/9) and joint pains(2/9). One patient was asymptomatic. ~66.7% patients had BM involvement, ~55.5% had autoimmune disorder and ~44.4% had splenomegaly. Lymphocytosis with presence of large granular lymphocytes was universal finding. Flow-cytometry findings showed predominance of T-lymphocytes with positive expression of CD3, CD8, CD2, CD57 while loss of CD5 and/or CD7. CD4, CD16 & CD56 were negative. Patients were treated with corticosteroids and methotrexate, if required. 6/9(66.7%) patients are stable and in remission, 1/9 died and 2/9 lost to follow-up.



Conclusion: Thus, T-LGL despite being a chronic leukemic disorder, has an indolent course, responds well to treatment with a prolong outcome

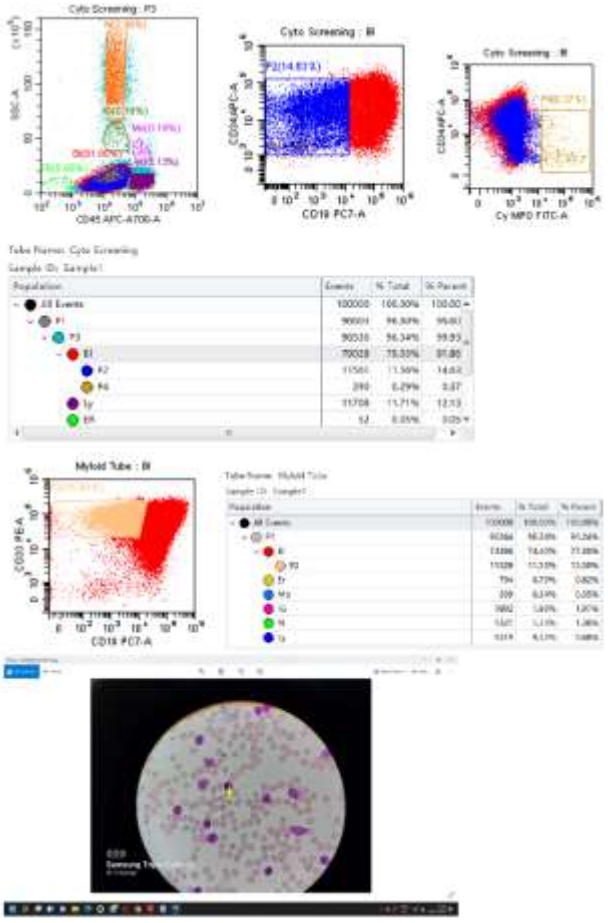
CP47 - Approach to a patient with Acute Leukemia in Mahavir Cancer hospital Patna, Bihar

Sarita Kumari, Sonal Jain, Amit Kumar, Khurshid Mallick, Manisha Singh

Mahavir Cancer Sansthan Patna

Introduction : Patient is four years old female complaint with fever on and off and weakness from one month. Hb- 3.3gm/dl; TLC- 66240/ul; Plt count- 24000/ul. Peripheral blood smear shows approximately 70% blast. Blast are large sized with relatively high N/C ratio, open chomatin. Majority showing basophilic cytoplasm.

Result: We identified ~81 of viable singlets gated in the blast window.Blasts are positive for CD19 and other B lineage marker, it also express CD34 and shows expression of HLADR and CD33. ~16% blasts are dim to negative for CD19. These blasts express moderate to bright CD33 and are positive for CD34. 0.9% of gated cells are positive for cyMPO and ~1 % are positive for CD64. Negative markers Blasts are negative for cyCD3, CD117, CD20, CD13 I



Auer Rod

Interpretation: The sample analysed shows predominant blasts of B lineage. However, in view of a proportion of blasts that are dim to negative for CD19(~16%), presence of very small proportion of MPO expressing cells (~0.9%), there is a strong possibility of Mixed Phenotype Acute Leukemia (MPAL B/Myeloid). Few blasts shows auer rods morphologically. Thus confirming the presence of Myloid blasts. Impression- Mixed Phenotype Acute leukemia (B- Myloid). Based on the flowcytometry findings and presence of auer rods.

Conclusion: The immunophenotyping by flow cytometry as an auxiliary method and in correlation with morphological findings it can make the diagnosis of acute leukemia more specific.

THE DIFFERENCE OF STANDARDIZATION

The BD FACSLyric™ system's on standardization help maximize compatibility, interoperability, repeatability and quality. Through the unique features of the BD FACSLYric flow cytometer, data is reproducible over time and across instruments, assays are easily portable between instruments and the workflow is simplified. Standardize your assay results with the BD FACSLyric system now. Learn more at **bdbiosciences.com/go/facslyric**

bdbiosciences.com

BD Life Sciences - Biosciences, Becton Dickinson India Private Limited, 6th Floor, Signature Towers-B, South City-I, Gurugram - 122001, Haryana, India. bdbindia@bd.com

BD

We thank our

Industry Partners

BD

BECKMAN COULTER
Life Sciences

sysmex | Together for a better healthcare journey

Star Biomed

premas life sciences

Dr Lal PathLabs
India's leading and most reputed diagnostic chain

For Their Support

sysmex | Together for a better healthcare journey

A New Vision for Flow cytometry

Embracing Automation to empower your laboratory

FLEXIBILITY	AUTOMATION	PERFORMANCE	DATA MANAGEMENT
SYSMEX REAGENTS	PS-10 SAMPLE PREPARATION SYSTEM AUTOMATED CELL WASH	XF-1600 FLOW CYTOMETER	SOFTWARE SOLUTIONS

- **Monoclonal Antibodies**
  Design a perfect panel for your diagnostic needs with Sysmex CyFlow monoclonal antibodies
- **PS-10 Sample Preparation System**
  Automated sample preparation with cell washer for increased flexibility (IVD approved)
- **XF-1600 Flow cytometer**
  Experience an exceptional performance with 3 laser 10 fluorescent parameter Analyser
- **Software Solutions**
  Improved software solutions for data analysis and reporting

www.sysmex.co.in