SOUVENIR

12th Annual Meeting of the
The Cytometry Society of India

www.tcs.res.in

9th -12th October 2019

Organized by

Department of Hematology,
Sanjay Gandhi Post Graduate Institute of Medical Sciences
Lucknow, Uttar Pradesh
India

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Faculty for the Conference

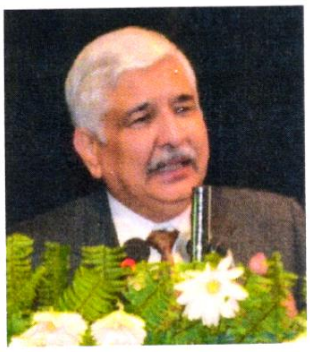
Image	Name	Designation
	Dr. Maryalice Stetler-Stevenson	NIH, Bethesda, United States
	Dr Francis Lacombe	Univ of Bordeaux, France
	Dr Sunil Kumar Arora	PGIMER, Chandigarh
	Dr Sumeet Gujral	TMH, Mumbai
	Dr Krishnamurthy H	NCBS, Bengaluru
	Dr Manisha Madkaikar	NIIH, Mumbai
	Dr Amita Aggarwal	SGPGI, Lucknow
	Dr P G Subramanian	TMH, Mumbai

Faculty for the Conference

Image	Name	Designation
	Dr Urmi Chatterji	University of Calcutta, Kolkata
	Dr Ritu Gupta,	AIIMS, Delhi
	Dr Anil Handoo,	BLK Hospital , Delhi
	Dr ManUpdesh S Sachdeva	PGIMER, Chandigarh
	Dr Vainav Patel	NIRRH , Mumbai
	Dr Prashant Tembhare	TMH, Mumbai
	Dr Prafullakumar Tailor	NII, Delhi
	Dr Namrata Punit Awasthi	RMLIMS, Lucknow
	Dr Kunal Sehgal	Sehgal Path Lab, Mumbai

Message

Professor Rakesh Kapoor,
Director,
Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow
U.P. India



It gives me great pleasure to know that the *Department of Hematology* is hosting the 12th Annual conference and workshop of The Cytometry Society (TCS), India from 9th October -12th October 2019. It is a matter of great pride to organize this event, in association with the Cytometry Society of India. I extend my hearty welcome to all the faculty members and delegates participating in this reputed conference & workshop.

The department of hematology was founded in the year 2003 under the headship of Dr. Soniya Nityanand, with an Objective to create a center of excellence for providing medical care, educational and research facilities of high qualities in the field of hematology. Since its inception, the department has created a niche for itself and achieved many goals. The department has a composite advanced laboratory and clinical wing to deal with patients suffering from various hematological disorders. I am proud to announce that it is a major referral centre in Uttar Pradesh where patients of blood cancer are aptly diagnosed using multiparametric flow cytometry and managed in accordance. Stem cell research is undoubtedly one of the key highlights of the department, for which it has successfully established a Stem Cell Research Unit for translational work on stem cells, out of the research funds sanctioned by the Dept of Biotechnology, Govt of India.

On this special occasion, we have a panel of national and international experts in the field of flow cytometry, who will deliberate on its various aspects and implications in today's era. I wish the department team a huge success in their endeavor to enrich the knowledge of the participating delegates.

Prof. Rakesh Kapoor

Message

Dr P.G. Subramanian
President
The Cytometry Society, India

It gives me great pleasure to invite you all for the 12th annual meeting of The Cytometry Society of India at SGPGI, Lucknow. The field of Cytometry is progressing into mainstream usage for various clinical and research application. This year's annual meeting emphasizes on bringing high dimensional data analysis techniques closer to routine usage. Dr. Francis Lacombe will be explaining the simplification of complex analysis of high dimensional data generated by cytometers of ever-increasing number of parameters. Dr Maryalice Stetler-Stevenson, is an expert faculty from National institute of Health, Bethesda, USA who will be talking about monitoring in Hemato-oncology after treatment with Cellular Immunological therapies that are emerging and are likely to be available in our country in the near future.

SGPGI being a centre doing Immunology work in India, we have sessions on Immunology related to studying Immune dysregulation. There is a session on recent emerging field of role in cell therapies and developments in identification of stem cells and other cell subsets using cytometry. My special thanks to Dr. Rakesh Kapoor, Director SGPGI for helping immensely the Cytometry Society by hosting the Annual meeting. I also thank Prof. Soniya Nityanand, Head, Dept of Hematology, Dr. Khaliqur Rahman and Dr. Ruchi Gupta for their excellent efforts in organizing this year's meeting in Lucknow.

Message

URMI CHATTERJI
SECRETARY, BASIC SCIENCES
THE CYTOMETRY SOCIETY, INDIA

It is a indeed a wonder that a technology invented almost five decades back has changed remarkably little in its fundamentals. A central challenge in the biomedical sciences has been to identify the distinct lineages and phenotypes of the specialized cells in organ systems, and track their molecular evolution during differentiation. Flow cytometry has since become an invaluable tool of biological research as well as clinical diagnostics, and its application has been crucial to innumerable advances in immunology and cell biology, and for understanding diseases such as AIDS and cancer. It has surpassed other key technologies and its application continues to expand, ranging from single-cell to cell population analysis. Approaching the norms of conventional flow cytometry, the systems also help in a deeper understanding of the advantages gained from the emerging field of nanoparticle technology. A powerful tool for interrogating complex biological questions at the forefront of biomedical and life science research, demands of today's investigators led to creation of smaller and more powerful instruments that are affordable and easier to use.

The Cytometry Society for the last few years have attempted to create awareness and inculcate a sense of technical advancement through organizing meetings and workshops, which emphasize that isolation and separation of single cells can be achieved, not only for detection of disease states, but also for therapeutic purposes. Cell sorting technology has paved the path for identifying single cells and studying various parameters displayed by the cell. Stem cell research would have not been possible without the advent of this wonderful technology. Flow cytometry not only allows for detection of apoptosis or intracellular antigens or even membrane potential, it can also perform specific recognition of cell surface antigens, membrane fluidity, protein modifications and chromosome analysis, thereby presenting a wide arena for different research ideas, thoughts and their implementation. This years' theme being "Flow Cytometry - Multi-Dimensional & Multi-Faceted" further emphasizes the versatile impact of this area of science and technology. I wish the local organizers great success for the 12th Annual Conference and Workshop and hope everyone enjoys 'flow'-ing!!!

Message

Prashant Tembhrae
General Secretary – Clinical Sciences
The Cytometry Society India

Dear Friends

Greeting!

As a general secretary – clinical, I extend a warm welcome to International, National Faculties and my fellow delegates from the various parts of India as well as abroad to the 12th Annual TCS meeting in Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, UP. Flow cytometry is a multidimensional dynamic technique that allows in-depth single-cell analysis. It has been contributing extensively in the field of basic and clinical research as well as clinical management of a broad range of diseases. In the last 2-3 decades, we have seen the vertical growth of this technology from basic two-color to 30-color highly advanced, next-generation cytometry. The field has shown a huge advancement in not only the spectrum of colors but also the depth of sensitivity matching to that of next-generation molecular techniques. In such an exciting era of flow cytometry, it is highly desirable to keep up our learning and share our experiences. The Cytometry Society (TCS) meeting provides a pleasant platform to talk, learn, share and collaborate with our colleagues as well as to meet and learn from international experts. TCS has been trying to accommodate as much as interesting topics possible in the various applications of flow cytometry ranging from the basics for the beginners to the recent advances in the flow cytometric assays like immune monitoring, MRD and RNA-flow cytometry. I can proudly say that the scientific contents and academic environment of TCS are improving every year and no less than any international cytometry meetings. With many valuable suggestions from TCS members and experiences from past meetings, the executive committee (EC) and organizing committee (SGPGI, Lucknow) has come up with an impressive program with a special focus on the current hot topic “MRD in acute leukemia and multiple myeloma”. I hope, you will find this meeting enriching your knowledge and will enjoy your stay in the ‘City of Nawabs’ and the ‘City of Tehzeeb

Message

Prof Soniya Nityanand,
Organizing Chairperson & Head, Dept of Hematology
SGPGI, Lucknow

It is an honor that the Cytometry Society of India (TCS) have identified the Dept of Hematology, SGPGI, to organize the 12thAnnual meeting of the Cytometry Society. In today's era, a Flow Cytometer is a life-line for any clinical Hematology Dept and a vital tool for the diagnosis, prognosis and monitoring of MRD of hematological disorders. Its application is now not restricted to malignant hematological diseases, but it has also been proven to be an essential component for work up of patients with various benign disorders and immunological diseases.

The Dept of Hematology at SGPGI is a composite department with both clinical and laboratory components and a major referral center of the State of UP and neighboring States, for patients with hematological disorders and the only center of UP performing Hematopoietic Stem Cell Transplants. The Dept is routinely performing multi-parametric flow cytometry in the immunophenotyping of hematological disorders and has gained considerable experience in this field over the years. The Dept is also deeply engaged in stem cell research and therapy for hematological as well as non-hematological disorders and has been funded by the Dept. of Biotechnology (DBT), Govt. of India, for infrastructure development under the DBT scheme of "Establishment of Centers of Excellence in Cell Based Therapy".

Keeping in pace with the new advances in the field of flow cytometry, the goal of the department is to share its experience and emanate knowledge by bringing together experts from the country as well as abroad to create a unique experience of learning and interacting with these experts through case discussions, data presentations, trouble-shooting etc.

The agenda is to conduct more such workshops in the years to come to fulfill our goal of sharing knowledge and expertise in the applications of flow cytometry in clinical medicine and stem cell research.

Message

It gives us great pleasure and honor to organize the prestigious 12th Annual Meeting of the Cytometry Society of India at our institute from 9th -12th October 2019. We are thankful to The Cytometry Society for identifying our center and providing the necessary support for the workshop. We would like to extend a warm welcome to all the National and International faculty members and delegates who have come from all over the country to participate in this prestigious event. We are particularly thankful to our faculty from abroad for their participation and sharing their expertise and knowledge.

The aim of this workshop is to bring together experts in the field of flow cytometry for disseminating knowledge and wisdom. The program encompasses a Lecture based teaching for the first two days, followed by the four parallel Workshops. The lectures and Workshops are planned in a fashion to cover all major clinical and research aspects of flow cytometry and its application in different disease states. The wet labs will give the participants a wonderful platform to learn, discuss ideas and exchange experiences with our eminent faculties. The knowledge imparted to the students will be a very useful investment in the field of flow cytometry, as they will transfer the same to the laboratories in their own centers.

We are indebted to our colleagues and the entire departmental team for their efforts in organizing the event. We wish that the participants will whole-heartedly enjoy the scientific feast that lays ahead in these 4 days. We hope your stay is comfortable and memorable and the delegates get a taste of the famous Awadhi cuisine and heritage of Lucknow.

Dr Ruchi Gupta
Organizing Secretary

Dr Khaliqur Rahman
Organizing Secretary

12th Annual TCS meeting and Workshop
Detailed Program Outline
Conference (9th -10th October 2019)

DAY 1 (09.10.2019) (Main Auditorium)			
Topic	**Time**	**Speaker**	**Chairperson**
About TCS- An Introduction	09:00 to 09:05	P G Subramanian	Rajesh Kashyap Amar Dasgupta
Newer Tools for FCM data analysis in hemato-oncology	09:05 to 09:45	Francis Lacombe	
Residual disease monitoring in the era of targeted /CAR T cell Therapy	09:45 to 10:25	M. Stetler-Stevenson	
INAUGURATION	10:25 to 10:50		
High Tea	10:50 to 11:20		
PARALLEL SESSIONS **(MAIN AUDITORIUM, LECTURE THEATRE 1 & 2)**	**11:20 TO 13:00**		
BASIC HEMATOLOGY – Lecture Theatre- 1	**Time**	**Speaker**	**Chairperson**
Role of FCM in AML- Case based Discussion	1120-1145	P G Subramanian	Ashutosh Kumar Seema Sharma Pradyumn Sngh
Role of FCM in ALL- Case based Discussion	1145-1210	Ritu Gupta	
Role of FCM in B CLPD- Case based Discussion	1210-1235	Kunal Sehgal	
Role of FCM in T CLPD- Case based Discussion	1235-1300	Sumeet Gujral	
ADVANCED HEMATO_ONCOLOGY -Main Auditorium			
Advances in FCM based PNH screening	1120-1140	Khaliqur Rahman	Sanjay Mehrotra Manjula Murari
FCM as a diagnostic tool in myelodysplastic syndrome	1140-1210	M. Stetler Stevenson	
Newer markers in B ALL MRD analysis	1210-1235	Gaurav Chatterjee	
FCM analysis of T ALL MRD: Case based discusiion	1235-1300	Prashant Tembhare	
BASIC SCIENCE RESEARCH – Lecture Theatre 2			
Defects of Immune tolerance	1120-1150	Amita Aggarwal	CP Chaturvedi Swasti Tiwari
Immune cells during parasitic Infection	1150-1220	Mrigank Srivastava	
Flow Cytometry of Microparticles/Exosomes	1220-1250	Vikrant Bhor	
Lunch and Poster Viewing	13:00-14:00		
Selected Oral Paper presentation- 5 each (Basic Science& clinical)	14:00 to 15:20		M Stetler Stevenson Amar Dasgupta Urmi Chatterji Vainav Patel
Best Published Paper (TCS Annual Award Recipient)	15:20 to 15:50		
Product Overview and New Launches- SYSMEX	15:50 to 16:00	Shruti Iyer	
Tea Break and Poster Viewing	16:00 to 16:20		
Advances in Cytometry and our Understanding of Immunity	16:20 to 16:35	Keefe Chee	Soniya Nityanand Sunil Arora
Mono and Polyfunctional T cells: Lesson learnt from HIV pathogenesis	16:35 to 17:00	Vainav Patel	
Excerpts from Cyto 2019- Annual Congress of ISAC	17:00 to 17:25	Uttara Chakraborty	
Quality assurance and good cytometry practices.	17:25 to 17:45	Anil Handoo	
EC Meeting	17:35 to 18:15		
Conference Dinner (Hobby Centre)	20:00 to 22:30		

12th Annual TCS meeting and Workshop
Detailed Program Outline
Conference (9th -10th October 2019)

DAY 2 (10.10.2019) (Lecture Theatre 1)			
Topic	**Time**	**Speaker**	**Chairperson**
Consensus guidelines for Multiple Myeloma MRD analysis & reporting	08:30 to 09:05	M. Stetler-Stevenson	P G Subramanian Ritu Gupta Sunil Arora
Multicentre harmonization in FCM analysis and its impact on MRD	09:05 to 09:40	Francis Lacombe	
Cancer Stem cells: Isolation and characterization	09:40 to 10:00	Urmi Chatterji	
Immune Dysregulation in malignant disorders	10:00 to 10:20	Gurvinder Kaur	
Immune Dysregulation in chronic viral Infections	10:20 to 10:40	Sunil Kumar Arora	
Tea Break and Poster Viewing	10:40 to 11:00		
Mass Cytometry: A step ahead of conventional Flow cytometry	11:00 to 11:35	Pavanish Kumar	Nuzhat Husain Vainav Patel Riaz Mehdi
Role of Flow Cytometry in Immunodeficiency Disorders	11:35 to 11:55	Manisha Madkaikar	
Flow Cytometry in Non neoplastic Haematology.	11:55 to 12:15	ManUpdesh S. Sachdeva	
Flow Cytometry in Non haematological neoplasms	12:15 to 12:35	Namrata Punit Awasthi	
DURA Innovations: Standardize with the expert, adopt high content Cytometry without worries	12:35 to 12:50	Shankar Pattabhiraman	
Lunch Break and Poster Viewing	12:50 to 14:00		
PARALLEL SESSIONS (LECTURE THEATRE 1 & 2)	**14:00 TO 15:15**		
Interesting Case Presentation- Lecture Theatre-1	14:00-15:15		
Non MRD cases: 06 MRD Cases: 03			
BASIC SCIENCE RESEARCH- Lecture Theatre -2	**Time**	**Speaker**	**Chairperson**
Identification and characterization of Mesenchymal Stem cells	14:00 -14:20	Soniya Nityanand	Dr Urmi Chatterji Gurvinder Kaur
Role of Flow Cytometry in Microglia/Macrophages characterization and Response	14:20-14:50	Alok Kumar	
Isolation and characterization of Cord blood Stem cells	14:50-15:10	Shobhita Katiyar	
GB Meeting and Valedictory	15:15 to 16:00		
Tea/Coffee	16:00 onwards		

12th Annual TCS meeting and Workshop

Workshop 1: Fundamentals of Flow Cytometry Workshop

Venue: I block, Dept of Hematology, SGPGI

11th – 12th October 2019

Host Co-ordinators: Dr Dinesh Chandra/Dr Sonal Seth/Manoj K Sarkar/Kaushal Kumar

Day 1 (11.10.2019)			
Time	**Topic**		**Faculty**
0900 - 1000	Principles of Flow cytometry		H Krishnamurthy
10:00-11:15	Know Your Instruments (In Batches)		Application Scientists from different companies
11:15-11:30	Tea Break		
11:30-12:15	Instrument Set up & Optimization (demo)		Sumanta Basu
12:15 -13:15	Sample Preparation (for Antibody titration and Compensation exercise)		Sumanta Basu/Manoj Sarkar/Vinay Gupta
1315-1400	**Lunch**		
1400-1500	Antibody Titration (Theory and demo)		Paresh Jain
1500-1600	Spillover, Compensation and Multicolor panel design (Theory)		Sumanta Basu
1600-1615	Tea break		
1615-1730	Demo of Compensation and Panel Design		Sumanta Basu Vinay Gupta
Day 2 (12.10.2019)			
0900-1000	Principles of leukemia panel construct, staining protocols and workflow (For Clinical)		Paresh Jain
	Parallel Session (1000 to 1630)		
1000 - 1300	**Basic Science**	**Clinical**	
	Microvesicle/Exosomes studies (Dr Vikrant Bhor)	Leukemia/ Lymphoma Staining* and acquisition (Dr Paresh Jain)	
1300-1400	**Lunch**		
1400-1630	Cell Cycle and DNA Ploidy Analysis (Dr H Krishnamurthy)	Leukemia/ Lymphoma Analysis (Dr Paresh Jain)	
1630-1700	Discussion, Evaluation and wrap up		

12th Annual Conference and workshop

Workshop -2: Advance Hemato-oncology Workshop

Venue: e-Tumor Board room, 2nd floor, Telemedicine Auditorium

11th – 12th October 2019

Host Co-ordinators: Dr Ruchi Gupta/Dr Mousumi Kar/Dr Manish K Singh

Day 1: 11.10.2019 (Friday)		
Time	**Topic**	**Speaker**
9:00-9:05	Introduction to workshop	Dr Khaliqur Rahman
9:05-9:35	Clinical relevance of MM MRD	Dr Ritu Gupta
9:35-10:10	Guidelines for MM MRD	Dr M Stetler-Stevenson
10:10-10:30	Tea break	
10:30-11:10	Concepts in Flow Cytometric MM MRD	Dr M Stetler-Stevenson
11:10-11:40	Template development for MM MRD	Dr Maryalice Stetler-Stevenson
11:45-12:10	MM MRD standardization: Experience from AIIMS	Dr Ritu Gupta
12:10-12:35	MM MRD standardization: Experience from TMH	Dr Prashant Tembhare
12:35- 13:15	Lunch	
13:15 - 14:30	Live case analysis (3 cases)	Dr M Stetler-Stevenson
14:30- 15:00	Live case analysis (1 cases)	Dr Ritu Gupta
15:00-15:20	Tea break	
15:20-15:50	Live case analysis (1 cases)	Dr Prashant Tembhare
15:50-16:50	Flow Cytometric Immunophenotyping of Pediatrics B ALL- An Indian Perspective with focus on Standardization on reporting across the labs	Dr PG Subramanian
16:50-17:15	Discussion and Conclusion	
Day 1: 12.10.2019 (Saturday)		
Time	**Topic**	**Speaker**
9:00-9:05	Introduction to workshop	Dr Khaliqur Rahman
9:05-9:30	Clinical relevance of AML MRD + TMH Data	Dr PG Subramanian
9:35-10:10	Flow Cytometric approach to AML MRD	Dr Francis Lacombe
10:10-10:30	Tea break	
10:30-11:00	Normal maturation of hematopoitic stem cells	Dr Prashant Tembhare
11:00-12:15	Application of novel software tools in AML MRD	Dr Francis Lacombe
12:15 to 12:40	ELN Guidelines for AML MRD	Dr Francis Lacombe
12:40: 13:10	Template development for AML MRD	Dr Prashant Tembhare
13:10 -13:50	Lunch	
13:50 - 15:00	Live case analysis (3 cases)	Dr Francis Lacombe
15:00 -15:25	Live case analysis (1 cases)	Dr Prashant Tembhare
15:25-15:45	Tea break	
15:45-16:15	Live case analysis (1 cases)	Dr PG Subramanian
16:15-16:45	Discussion and Conclusion	

12th Annual TCS meeting and Workshop

Workshop 3: Cell Sorting

Venue: I block, Dept of Hematology, SGPGI

11th – 12th October 2019

Host Co-ordinators: Dr C P Chaturvedi/Shobhita Katiyar

		11.10.2019 (day 1)	
	0930-1100	Basics of Cell Sorting	Swapnil Walke Rekha Gour
	1100-1300	Instrument setup and QC	Swapnil Walke
		LUNCH	
	1345-1515	4 way sorting using beads	Swapnil Walke Rekha Gour
	1515-1600	Post Sorting Purity analysis	Swapnil Walke
	1600-1630	Machine Shut down & QC	Swapnil Walke
		12.10.2019 (day 2)	
	0930-1100	Single cell preparation using Mouse spleen	Mrigank Srivastava
	1100-1300	4 way/2 way cel sorting, Post Sorting Purity	Mrigank srivastava
		LUNCH	
	1345-1515	Human lymphocyte Sorting	Swapnil Walke
	1515-1615	Plate Sorting	Swapnil Walke
	1615-1645	Machine Shut down and wrap up Evaluation	Swapnil Walke

12th Annual TCS meeting and Workshop

WS 4: Immunodeficiency

11th – 12th October 2019

Venue: Department of Clinical Immunology & Rheumatology

Host Co-ordinators: Dr Amita Agarwal

Time	Topic	Person
Day 1 - 11.10.19 (Friday)		
0900-10.00	Introduction to flow cytometry	H Krishnamurthy
1015-1040	Introduction to PID	Amita Aggarwal
1040-1120	Screening for PID and approach to diagnosis of SCID	Manisha Madkikar
1120-1145	***Tea Break***	
1145-1245	Lymphocyte subsets	Komal
1245-1315	Immunoglobulin levels by nephelometry,	Komal, Lalit
1315-1400	***Lunch***	
1400-1420	B cell Immunodeficiency: diagnosis	Amita Aggarwal
1420-1630	B cell subsets	Komal and Ankita
1630-1700	CD11/CD18 assay	Komal, Rajesh
Day 2 - 12.10.19 (Saturday)		
0900-0930	Phagocytic defects: approach	Manisha Madkikar
0930-1100	DHR assay	Komal and Rajesh
1100-1130	**Tea Break**	
1130-1215	HLH/MAS diagnosis	Manisha Madkikar
1215-1300	Perforin assay	Komal
13.00-14.00	**Lunch**	
1400-1630	Th17/Treg assay,sCD25 by ELISA	Komal, Ankita
1630-1500	QA session, Workshop evaluation	Amita Aggarwal

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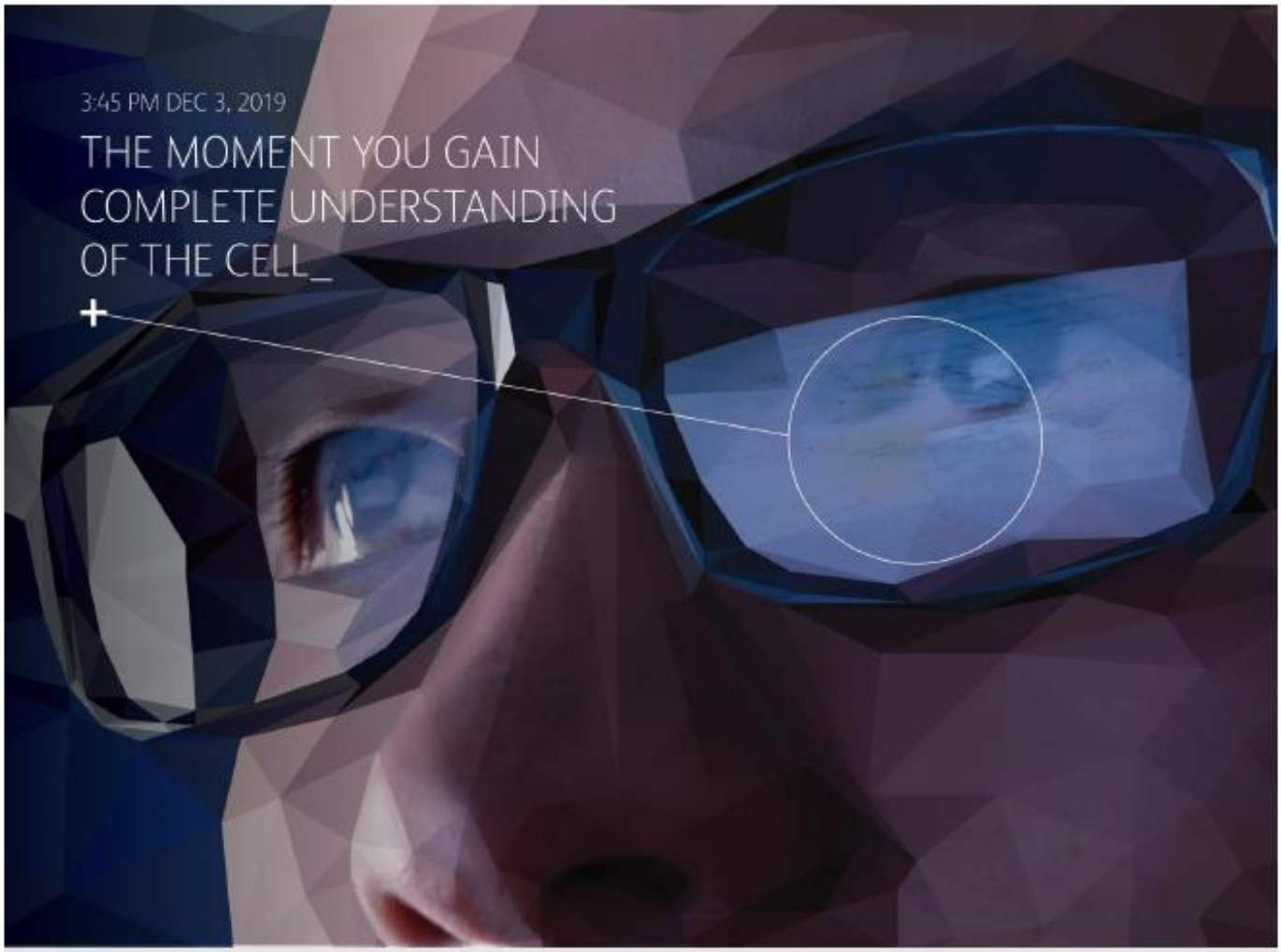

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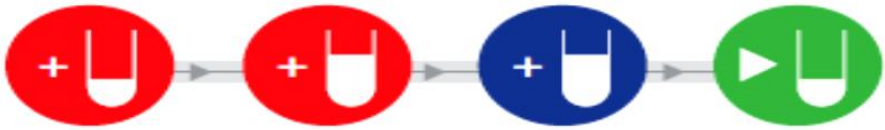

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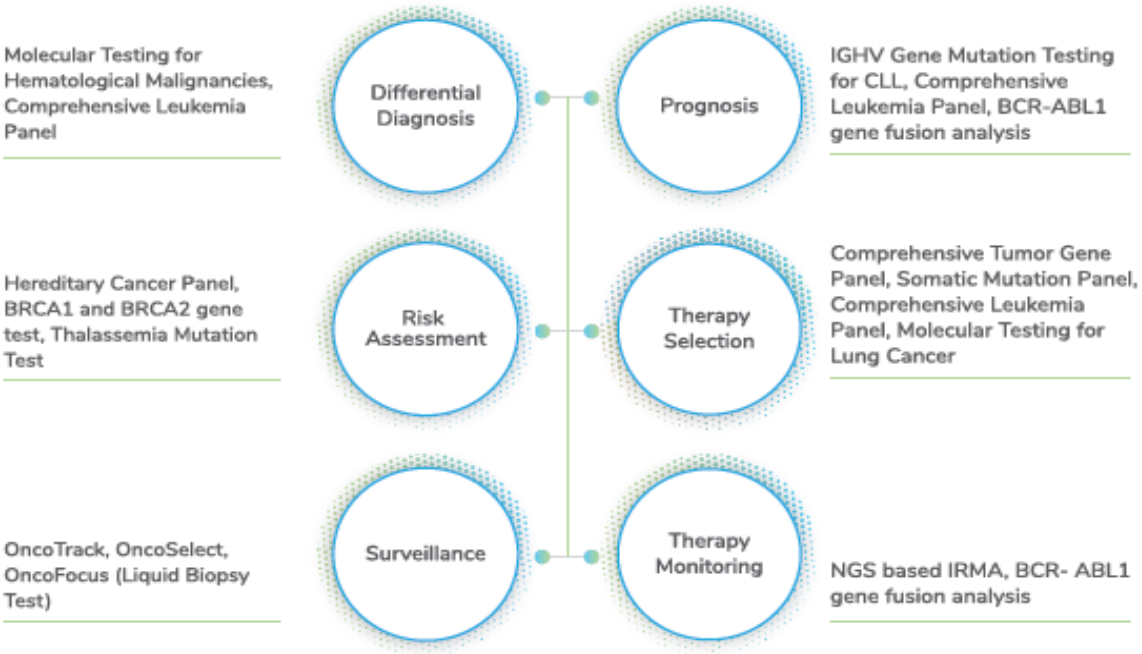

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Newer tools for FCM data analysis in hemato-oncology

Francis Lacombe

The exploration of human bone marrow (BM) is an important tool to understand and diagnose diseases involving altered hematopoiesis. Although morphological identification of cells with distinctive features still remains the basis of these analyses, more sophisticated methods appeared to be necessary, in particular flow cytometry. To assess reproducibility of immunophenotyping analysis between different laboratories and different flow cytometers prerequisites are required and we will present the so called “Harmonemia” process we developed in this perspective.

Many new multidimensional software (t-sne, Spade…) were designed for the analysis of big-data generated in mass cytometry (MS). Their application to classical multiparameter flow cytometry (MFC) is still seldom reported. FlowSOM, developed for MS within the Bioconductor open-source project, has been praised for its discriminative abilities and operator-friendly application. We will show a combination of FlowSOM and Kaluza® software to analyze human bone marrow (BM) in MFC. Antibody panels adapted for the diagnosis of acute leukemias were applied to normal BM (NBM) samples. providing new ways of dissecting normal hematopoietic differentiation. Reference MST of NBM were then merged with diagnosis and follow-up files from patients with acute myeloblastic leukemia (AML) or acute lymphoblastic leukemia (ALL), for unsupervised concomitant analysis. This outlined the immunophenotypic heterogeneity of AML and ALL and allowed a fine delineation of minimal residual disease.

The combination of automated sophisticated multidimensional representation of numerous subsets and their in-depth analysis by classical manual tools of FCM analysis is a truly revolutionary approach. These versatile new tools open the way for a new era of classical flow cytometry analysis in hematological malignancies.

Residual Disease Monitoring Post Targeted Therapies in ALL

Maryalice Stetler-Stevenson M.D., Ph.D.

Advances in antigen-targeted immunotherapies have made tremendous progress, particularly with CD19 and CD22 targeting in B-cell acute lymphoblastic leukemia (ALL). By circumventing inherent chemotherapeutic resistance and harnessing T-cell based killing, these treatments expand options for patients, particularly for those with relapsed or chemotherapy refractory disease. Based on their high remission induction rates, blinatumomab, a (CD19/CD3) bispecific T-cell engaging antibody, inotuzumab (CD22 immunotoxin), and CD19 chimeric antigen receptor (CAR) T-cells have received FDA approval for the treatment of ALL and CD22 CAR T-cell therapy is in advanced clinical trials. In the setting of such targeted therapies, CD19 and CD22 expression may be decreased or lost, compromising the use of these reagents for identification of abnormal blasts post therapy. CD19 and CD22 are often sequentially targeted which can result in modification of both antigens, i.e. CD19 and CD22 negative ALL can evolve. Flow cytometry is a cornerstone of minimal residual disease (MRD) monitoring post therapy in ALL. CD19 and CD22 are gating reagents that have been utilized for ALL MRD identification by flow cytometry, however, in the era of targeted therapy against these antigens novel approaches to MRD detection are called for. Strategies for flow cytometric monitoring for MRD in patients with ALL post anti CD19 and anti CD22 therapies will be discussed.

Monofunctional and Polyfunctional T cells: Lesson learnt from HIV pathogenesis

Vainav Patel

The presentation will explore and highlight the use of multi-colour immunophenotyping and flow cytometry in the identification of various cellular immune signatures associated with naive and therapy receiving states of HIV-1, HIV-2 and dual infection. Signatures delineating disease progression, non progression and immune restoration will be discussed with respect to T and Monocyte subsets. Functional data pertaining to T and monocyte activation, cytotoxic potential, polyfunctionality in antigen specific responses as well as regulatory and homeostatic distribution will be presented.

Excerpts from CYTO 2019- Annual Congress of the ISAC

UrmiChatterji

What started out as the Society for Analytical Cytology gradually metamorphosed over the last 43 years growing, expanding and diversifying in different domains of cytometry eventually evolving into the ISAC (International Society for Advancement of Cytometry). The annual multinational cytometry congress of the ISAC named CYTO has become one of the biggest international collaboration platforms for the advancement of the field of cytometry. CYTO 2019 was hosted in the city of Vancouver in Canada in June. It spearheaded the 34th Congress of the ISAC and showcased the field of cytometry to the community with the latest scientific discoveries enabling measurement of cells with more depth, more sensitivity and more robustly than ever before. The three emerging technologies like mass, image and genomic cytometry now widely used across new biological and clinical settings around the globe, addressed important research areas like cancer and blood disorders, stem cell research and therapy and detection of infectious disease pathogens. This year however, CYTO celebrated a revolution in single cell technology through its different sessions including the Plenaries, Frontiers, Tutorials and State-of-the-Art lectures. Starting from pioneering cytometrists, thought leaders to cutting edge technologists, CYTO 2019 was an eye-opener for all. Apart from the scientific sessions of all kinds, it was the leadership development initiatives designed by the ISAC under the IMISP and SRL Emerging Leader programs that paved way for the new generation emerging cytometrists to be a part of this meeting enhancing the scientific and leadership experiences of research leaders around the world. My talk would showcase excerpts from CYTO 2019 to discuss the latest discoveries and innovations in cytometry, with special emphasis on the leadership development programs and how this can lead to establishing strong collaborations between academia- industry-clinicians to take our own research disciplines to the next level as per International standards of cytometry.

Flow Cytometric Immunophenotyping of ALL- A Case Based Discussion

Ritu Gupta

Acute Lymphoblastic Leukemia (ALL) is the hematopoietic malignancy of B/T-lymphoid precursors. Multi-parametric flow cytometric immunophenotyping (FCMI) is the mainstay of diagnosis and post treatment response evaluation. In this lecture, 3 unusual cases of ALL which posed diagnostic challenge and/or dilemma in response assessment will be discussed.

Case I: An infant presented with fever and hyperleukocytosis. Bone marrow aspirate showed 90% blasts which were CD45+CD19+ and negative for all other lymphoid and myeloid markers. Extensive evaluation showed positivity for CD15 and NG2 as well as t(4,11) on cytogenetics. So, a final diagnosis of Infantile B-ALL with MLL-gene rearrangement was given.

Case II: A 9-year old child with 85% blasts on peripheral blood smear which on FCMI showed positivity for B-lymphoid markers. However, the expression pattern of various antigens on B-lymphoblasts was consistent with hematogones (CD45dim CD19+CD10+CD20htgCD38mod). At post-induction time point, bone-marrow FCMI showed hematogones, which posed diagnostic challenge as it was difficult to distinguish between leukemic lymphoblasts and hematogones.

Case III: An adolescent young male presented with fever and hyperleukocytosis. FCMI of the peripheral blood showed T-lymphoblasts with cCD3+CD7+CD5+. Post-induction bone marrow sample for MRD assessment showed 5% blasts which on FCMI showed an ETP clone (cCD3+CD7+CD5-CD34+CD10+CD33+).

In summary, diagnosis of ALL by FCMI may pose diagnostic dilemma occasionally, thus a multimodality approach combining extensive panel of antibodies with molecular and cytogenetic techniques are the pre-requisite for making correct diagnosis. The MRD assessment by FCMI is now being incorporated in clinical decision making process, we need to explore more aberrant markers to make a reliable MRD diagnosis in cases with hematogone pattern. Finally, a careful analysis and documentation of all minor clones and sublones at baseline is mandatory to analyse follow-up samples for MRD evaluation.

Newer markers in B ALL MRD analysis

Gaurav Chatterjee

Presence of measurable residual disease (MRD) by sensitive techniques, in patients otherwise in morphological remission, has been established as the singular most powerful factor for predicting relapse in B ALL patients. Multicolor flowcytometry (MFC) is a suitable method for MRD monitoring as it is a cheaper, widely available technique with rapid turn-around-time allowing for quick treatment modifications. However, even with the currently established methodologies for MRD evaluation, a subset of patients relapses despite being MRD negative.Therefore, careful addition of new markers and finetuning of existing MFC panels, in addition to acquiring a larger number of events, are imperative for improvement of MRD detection. Proper evaluation of a new marker including its aberrant expression at diagnosis and immunophenotypic stability at time-point of MRD evaluation is necessary before inclusion in routine panel. Recently, others and we have performed extensive evaluation of newer markers like CD24, CD44, CD123, CD72, CD73, CD86,CD200, CD25, CD66c and CD304 in B ALL MRD analysis. The current data indicates that CD73, CD86 and CD304 are helpful in a large proportion of cases for MRD detection and should be incorporated in routine B ALL MRD panel.

Approach to flow cytometric based diagnosis of B- cell Chronic Lympho Proliferative Disorders

Kunal Sehgal

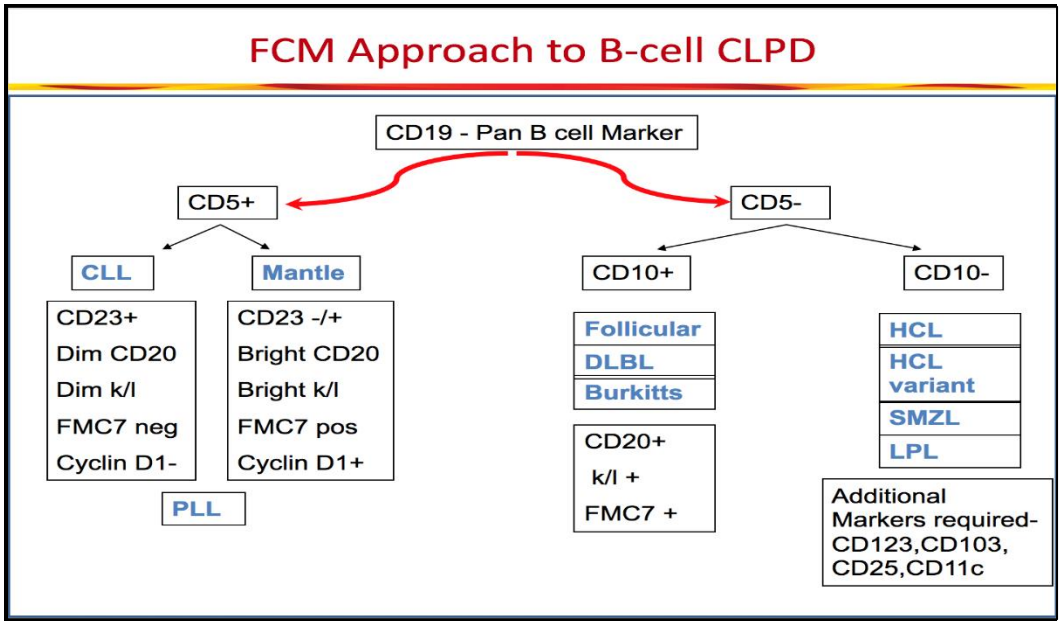
Diagnosis of Lymphomas and Chronic Lympho-proliferative disorders requires a multidisciplinary approach including -

- Adequate Clinical History and Examination
- Laboratory work up- CBC,ESR,LDH,B_2 microglobulin, etc.
- Radiological Evaluation- PET Scan, CT scan , etc.
- Molecular Studies
- Morphology – H & E
- Immunophenotyping- for diagnosis, subtyping, prognosis

Role of FCM in CLPD is as follows -

- Diagnosis of CLPD – Reactive vs. Lymphoma
- Typing and Sub classification
  - B cell CLPD
  - T cell CLPD
  - NK cell CLPD
- Prognostic markers
  - eg. CD38 and ZAP-70 in CLL

Diagram below describes the conventional approach to B- CLPD classification and the talk will discuss in detail a case based approach using the below common markers as well as discuss role of newer markers including CD200, CD43, CD180, CD43 and others for classification of common B- cell lymphomas



Flow Cytometry: A Diagnostic Tool in Myelodysplastic Syndromes

Maryalice Stetler-Stevenson M.D., Ph.D.

The myelodysplastic syndromes (MDS) are a heterogeneous group of clonal stem cell diseases that are characterized by cytopenias with progressive marrow failure and an increased risk of myeloid leukemia. Although bone marrow biopsy with concurrent cytogenetic studies have been the gold standard for the diagnosis of MDS, a significant number of patients have similar peripheral blood and bone marrow findings that make diagnosis and classification difficult. For this reason, laboratory scientists have applied flow cytometric immunophenotyping (FC) to the study of MDS. Early pivotal studies found immunophenotypic evidence of atypical granulocytic maturation and aberrant antigen expression in myelodysplasia. Later, multiparametric flow cytometric studies of the full spectrum of granulocytic, monocytic and erythroid immunophenotypic abnormalities definitively demonstrated that flow cytometry increases the sensitivity and specificity of diagnosis in MDS. Further studies demonstrated that FC is an independent prognostic indicator for MDS patient relapse post transplant and overall survival. Recent studies have focused on standardizing panels and analysis approaches to MDS diagnosis. FC methods to detect myelomonocytic and erythroid dysplasis and facilitate a diagnosis of MDS will be discussed.

Multicenter harmonization analysis and its impact on MRD assessment

Francis Lacombe

Flow cytometry is now necessaryfor the exploration of human bone marrow (BM) especially for diagnose diseases involving altered hematopoiesis. The development of monoclonal antibodies identifying hundreds of leukocyte differentiation antigens has been a major step which progressively led to the definition of sets of markers of interest for the various lineages of normal hematopoiesis. In an effort to produce consensual guidelines useful for accreditation procedures, the French Groupe d'Etude des Leucémies (GEIL) organized a series of workshops between 2010 and 2012. We first isolated from the European LeukemiaNet (ELN) mandatory markersthose with diagnostic value in acute leukemia and lymphoproliferative disorders. We chose to minimize the number of fluorochromes for a given specificity, whatever the panel involved, in order to better control costs and minimize errors in cocktail preparations. Since rigorous analysis strategies are also highly important, we chose to use CD45 in all tubes, in order to identify leukocyte subsets.To assess reproducibility of immunophenotyping analysis between different laboratories and different flow cytometers we developed a new concept called Harmonemia and published in Leukemia.It allowed an harmonization of photomultiplier (PMT) settings and used a staining of normal peripheral blood (PB) with a common antibody panel.TheHarmonemia project involved 23 instruments from Beckman Coulter (n= 16)and BD Biosciences(n= 7) in 17 laboratories for PMT settings. Ultimately, Harmonemia aims at working in lysis/ no wash protocols. In such settings, normal cells in the sample act both as calibrators and as controls. Autofluorescence provides internal calibration for PMT settings while intrinsic positive control is provided by proper labeling of cell populations targeted by the reagents used.

The Harmonemia process is involved in MRD detection inside the ELN group on MRD study in AML,and appears to be a prerequisite for application of new software like FlowSOM to MRD analysis in Acute leukemia. It can be applied both to Beckman Coulter ® and Becton Dickinson ® platform.

Consensus Guidelines MRD in Multiple Myeloma

Maryalice Stetler-Stevenson M.D., Ph.D.

New and effective treatments for plasma cell myeloma (PCM) are being developed at an unprecedented rate. As PCM therapies become more effective, assessing treatment efficacy according to MRD levels becomes increasingly important. Immunophenotypic complete response in PCM is shown to be one of the most relevant prognostic factors for patients undergoing autologous stem cell transplantation, as well as in non-transplant eligible patients treated with novel agents. For two decades MRD analysis in PCM has been a more sensitive approach to conventional response assessment and flow cytometry (flow-MRD) has been the preferred method used in clinical trials because of the following: flow-MRD with a standard panel is applicable to all patients; flow- MRD assays incorporate a quality check of the whole sample quaity (e.g. hemodilution); and flow-MRD assays are directly quantitative with the same limit of detection (LOD) and quantification in every case. Due to its increasing importance as a clinical test, standardization of flow cytometric PCM MRD testing of bone marrow aspirates is vital to ensure better and uniform assessment of response and clinical prognostication. In line with this, the International Clinical Cytometry Society (ICCS) and European Society for Clinical Cell Analysis (ESCCA) worked together to develop consensus guidelines for flow cytometric MRD testing in plasma cell myeloma. The specific guidelines for analysis and reporting will be discussed.

Cancer stem cells: isolation and characterization

Urmi Chatterji

Previous studies examining breast cancer tissues have demonstrated the presence of breast cancer stem cells (brCSCs) within the tumors which are radioresistant and chemoresistant. Stem cells were isolated and enriched from normal breast and triple negative human breast tumors, and MDA-MB-231 breast cancer cells, using flow cytometry and the expression of self-renewal markers, like Wnt, Sox-2, Oct4 and Nanog, were analyzed. A paclitaxel inhibition test was also conducted in order to detect resistance of brCSCs to this treatment. Contrary to the quiescent nature of cancer stem cells (CSCs) within a tumor, a gene profiler array revealed that phosphatidylinositol-3-kinase (PI3K), crucial for cell proliferation, differentiation and survival, was significantly up regulated. The effect of cAMP on PI3K and interplay of two of its regulators - PKA and PTEN was evaluated using a PDE4 inhibitor, Rolipram, and its efficacy was compared with Paclitaxel, a conventional anticancer drug. Analyses of interactions between cell death-related proteins revealed a fine-tuning between autophagy and apoptosis/necrosis of the CSCs in response to Rolipram. Rolipram-mediated downregulation of PDE4A levels in breast CSCs increases cAMP levels and PKA expression. Moreover, PKA-mediated upregulation of PTEN antagonized the PI3K/AKT pathway, and led to cell cycle arrest. mTOR being a downstream molecule of cAMP-PKA activation, shifted death of CSCs towards apoptosis and necrosis by reducing autophagy over time. Rolipram can therefore be used to modulate the cAMP/PKA/mTOR pathway in breast CSCs and effectively obliterate them within a tumor. These findings may have clinical implications to use PDE inhibitors like Rolipram in trial designs as a possible chemotherapeutic agent to effectively destroy the breast CSCs.

Immune dysregulations in chronic viral infections

Sunil K Arora, PhD, MNAMS

Advanced stage of chronic viral infections (HIV & HCV) is associated with severely immune-dysregulated state. Poor handling of infectious organisms during this state has been linked to functionally defective antigen presentation. The molecular mechanisms behind DC impairment are still obscure. Dysregulation in the DC functions is found to be associated with the expression of key regulators of cytokine signalling and some negative regulators of DC maturation.Critical evaluation of phenotypic and functional characteristics of circulating myeloid DCs (mDCs) in therapy-naive patients in chronic stage of diseases and on therapy longitudinally prior-to and after the start of therapy, reveals very interesting results. The ability of professional antigen presenting cells (the myeloid DCs) to respond to an antigenic stimulation is severely impaired in the patients with these infections showing recovery after a successful therapy in HCV, while only partial in HIV. The mDCs seem to have lost the capacity to mature in response to external stimulus and depict very poor allo-stimulation and reduced cytokine secretion even after TLR-4 mediated stimulation *ex-vivo*. On molecular scrutiny the cells show an imbalanced expression profile of many factors, which are known to influence the maturation capacity of mDC in negative of positive manner. An increased expression of negative regulatory factors like SOCS-1, SOCS-3, SH2-containing phosphatase (SHP)-1 and a reduced expression of positive regulators like Janus kinase(JAK)2 and Nuclear factor kappa-light-chain-enhancer of activated B cells(NF-κB)1. A functional recovery after siRNA mediated silencing of SOCS-1 in these mo-DCs confirms the role of this negative regulatory factor in functional impairment of these cells. Functionally defective state of DCs in the advanced stage of HIV-1 infection seems to be due to imbalanced expression of negative and positive regulatory genes. Whether this is a cause or effect of increased viral replication at this stage of disease, needs further investigation. The information may be useful in design of novel therapeutic targets for better management of disease.

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Systems immunology using Mass Cytometry identified structural rewiring of immune system in refractory epilepsy

Pavanish Kumar

Drug refractory epilepsy (RE) is a chronic neurological disease with varied etiology that represents a group of patients whose seizures do not respond to anti-epileptic drugs. In children, refractory epilepsy is often associated with significant disability and even mortality. The immune system may have role in seizure and epilepsy development, but the specific mechanisms of inflammation that lead to epileptogenesis and contribute to RE are unknown. Autoimmune encephalopathy (AIE), a group of acquired, often antibody-associated central nervous system inflammatory disorders, can certainly present with frequent seizures in affected children, but there is little if any data regarding such mechanisms specifically in pediatric RE.

Here, we used high dimensional mass cytometry to comprehensively study the immune system of pediatric patients with RE and compared their immune profile and function with patients with age matched autoimmune encephalitis (AIE) and healthy controls. Patients with RE and AIE displayed similar immune profiles overall, with changes in CD4+ and CD8+ T-cell subsets and an unbalance toward pro-inflammatory IL-17 production. In addition, patients with RE uniquely showed an altered balance in natural killer cell subsets. A systems level intercellular network analysis identified rewiring of the immune system leading to loss of inhibitory/regulatory intercellular connections and emergence of pro-inflammatory pathogenic functions in neuroinflammatory immune-cell networks in patients with AIE and RE. These data underscore the contribution of systemic inflammation to the pathogenesis of seizures and epileptogenesis and have direct translational implications in advancing diagnostics and therapeutics design.

Role of Flow Cytometry in Immunodeficiency Disorders

Manisha Madkaikar

Primary immunodeficiency diseases (PID) are clinically and immunologically heterogeneous group of disorders of immune system. Diagnosis of these disorders is often challenging and requiresidentification of underlying genetic defect complemented by comprehensive evaluation of immune system.Flow cytometry, with its advances in last few decades has emerged as an indispensable tool for enumeration as well as characterization of immune cells. Flowcytometric evaluation of immune systemnot only provides clues to underlying genetic defect in certain PIDs and helps in functional validation of novel genetic defects,but is alsouseful in monitoring immune responses following specific therapies. India has seen significant progress in the field of flow cytometry as well as PID over last one decade in India. Currently, in India, there are seven Federation of Primary Immunodeficiency Diseases (FPID) recognized centers across India including two IndianCouncil of Medical research (ICMR) funded centers of excellence for diagnosis and management of PIDs. These centers offer comprehensive care for PIDs including flow cytometry-based evaluation. The key question which always remains is how one selects from the wide array of flow cytometry-based tests available and whetherall these tests should be performed before or after the identification of genetic defects. This becomes crucial especially when resources are limited and patient has to pay for the investigations. I will share some of our experiences based on evaluation of large cohort of severe combined immunodeficiency, chronic granulomatous disease, hemophagocytic lymphohistiocytosis and Mendelian susceptibility to Mycobacterial diseases and the lessons learnt for optimum use of this powerful technology for diagnosis of these disorders.

Flow cytometry in Non hematological neoplasms

Namrata Punit Awasthi

In the 1960s, Flow Cytometry (FCM) was introduced as an analytical technique with an ability to measure various characteristics of single cells in suspension following excitation with a light source. The clinical applications of flow cytometry in oncology started with the measurement of DNA content for the evaluation of both ploidy and cell cycle profile as potential prognostic parameters in the majority of human solid cancer types. The availability of monoclonal antibodies widely broadened the spectrum of clinical applications of this technique, which rapidly became a fundamental tool for the diagnosis and prognosis of malignant hematological diseases. With further advancement in this technology, there have been attempts to utilize this fascinating machine for varied applications on solid tumors or non hematological malignancies. In principle, any sample which can flow (as single cell suspension) can be flowed.

1.Immunphenotyping in solid tumors: Histopathology with immunohistochemistry (IHC) remain the primary diagnostic modality for solid tumors, however FCM can be useful in serous cavity effusions in patients with history or a suspicion of an epithelial neoplasm. Many antigens have been tested but EpCAM (Epithelial cell adhesion molecule, Ber EP4, CD326 and MOC31) has emerged as the best marker to detect epithelial cells by FCM [1]. EpCAM based FCM has been reported to have a greater sensitivity than cytology and can provide rapid confirmative immunophenotyping in cases that are positive by morphology obviating the need for IHC[1]. As the rare malignant cells can be detected by FCM due to analysis of larger volume of fluid, these cases can be labeled positive with a greater degree of confidence as compared to cytology wherein these cases are best labeled negative or suspicious. Ashort turnaround time is a clear advantage.

A small proportion of epithelial neoplasms which do not express EpCAMcan lead to a false negative result. EpCAM-positive events have also been found in negative serous effusions and lymph nodes in very low numbers giving a false positive diagnosis. Addition of more antibodies to the panel will help in improving the sensitivity and specificity of this technique.

FCM is routinely performed on lymph nodes to rule out lymphoma and an additional analysis for EpCAM-positive cells for an epithelial malignancy or a pediatric cancer panel can be added for flow cytometric characterization of the involved lymph node. A pediatric cancer FCM panel that was used on a wide variety of tissue samples revealed distinct immunophenotypes for neuroblastoma (CD56hi/GD2+/CD81hi), primitive neuroectodermal tumors (CD271hi/CD99+), Wilms' tumor (CD56+, multiple populations), rhabdomyosarcoma (MYOD1+/myogenin+), germ cell tumors (CD56+/CD45–/NG2+/CD10+), and hemangiopericytomas (CD45–/CD34+) [2]. Although definitive diagnosis requires morphological assessment, the advantage of flow cytometric analysis in this situation is that it provides a rapid, same-day immunophenotypic characterization[2,3]. However, in a resource poor setting, this application might not sound feasible.

Flow cytometric analysis shows a high sensitivity and specificity for the diagnosis of carcinomatous meningitis [4,5]. The utility of FCM lies in the ability to immunophenotype rare cells. Rapid degeneration of CSF specimens, a low volume and cellularity, blood contamination, and nonspecific background fluorescence are technical issues that need to be resolved [6]. It is suggested that at least 5 ml of fluid and 100 cells need to be analyzed, with a cluster of at least 10 events in order to consider a result positive.

2.DNA Ploidy and cell cycle analysis: The analysis of nuclear DNA content in terms of ploidy and cell cycle profile has been performed by FCM for more than 25 years. Initially it was applied to hematological tumors but with development of reliable tissue disaggregation and staining techniques, with increasingly sophisticated

multiparametric analyses and dedicated software, it was extended to almost all solid cancers like prostate, breast, colon, bladder, cervical etc. Investigators have focused on the correlation of tumor DNA ploidy and proliferative activity (S Phase fraction) with known prognostic factors, in particular clinical stage, histologic grade and patient clinical course. Although these appear promising, they do not always correlate with classical clinical-pathological parameters or prognosis. Studies performed on archival material (formalin-fixed, paraffin-embedded tumor specimens) suffer more than those performed on fresh tissues. The resolution of DNA histogram from archival tissues is usually lower than that obtained from fresh tissues. Enzymatic digestion required to produce single cell suspension results in variable amounts of subcellular debris which is superimposed to the DNA histogram and may lead to overestimation of S-phase fraction. External diploid standards cannot be utilized to determine which G0/1 peak represents the diploid subpopulation because tissue fixation leads to variations in fluorochrome binding. All these technical issues combined with limitations in the design of the study lead to lack of concordance between various studies. In summary, despite early optimism, the clinical applications of DNA content analysis by FCM did not show a real impact on the diagnosis of neoplastic diseases or in the determination of prognosis of cancer patients [7,8,9].

3. **'Rare event' analysis**: A cell is considered 'rare' when the number of that specific subpopulation represents <0.001% of the entire population [7]. One of the major advantages of FCM comes from its ability to obtain information from single cells identified inside a heterogeneous cellular population due to its ability to analyze extremely large number of cells. Some examples of rare event analysis that can be studied by FCM include the study of the minimal residual disease (MRD) in hematological malignancies, the detection and quantification of circulating tumor cells, blood-circulating dendritic cell subsets, circulating endothelial cells and their progenitors, and cancer stem cells.

3.1 Circulating tumor cells: In the last 10 years, circulating tumor cells (CTCs) have been widely studied as new biomarkers, because they provide a non-invasive source of tumor material. CTCs have been used in various studies for (1) achieving early diagnosis; (2) dynamic monitoring of treatment response (3) evaluating relapse and metastatic risk; (4) studying genetic evolution of the tumor and its heterogeneity (5) identifying possible therapeutic targets and assessing drug resistance [10]. CTCs are extremely rare and occur in peripheral blood in a ratio of about 1 CTC per 10^6-10^8 leukocytes. Enrichment and their subsequent detection is hence a challenging issue. Since CTC can, in a non invasive way, provide clinically relevant information in cancer follow-up there has been a concerted effort to develop sensitive platforms to isolate, enrich, and analyse CTCs. Flow cytometry was one of the first techniques used for the detection of blood CTCs. It is an excellent tool for this purpose, because it possesses the ability to easily recover viable cells with high purity that are suitable for downstream molecular analysis. In recent years, different automated cytometry techniques have been employed to further subdivide CTCs according to tumor origin and proliferative/invasive capacity, and FCM remains promising in this field. Prognostic value of CTC enumeration has been shown in several types of epithelial tumors notably breast, prostate, colorectal cancer, pancreatico-biliary and hepatocellular. However, as a general consideration, although CTCs are already used in clinical trials as a possible biomarker, their routine clinical utility is still under active investigation.

3.2 Circulating Dentritic cells: Dendritic cells (DC) are specialized phagocytes endowed with an ability to sense small changes in the tissue microenvironment and activate adaptive immune responses, whilst maintaining immunological tolerance. They are crucial antigen-presenting cells and play a primary role in antitumor immune response by controlling the initiation of the T cell-dependent immune response. Two peripheral blood DC subsets have been identified on the basis of their CD11c expression: The CD11c-negative (CD11c-) DCs (that express high levels of CD123), plasmacytoid/lymphoid-derived DCs (pDCs), and the CD11c+/CD123-cells,

myeloid-derived (mDCs) [7]. In the last several years, several FCM studies have focused on the determination of the number and of the functional alterations of DCs and their subsets in different type of human cancers like breast, lung, melanoma etc. in an attempt to correlate them with prognosis and patient outcome. pDCs act differently depending on the microenvironment they encounter and can therefore have an inhibitory immune activity or antitumor activity. The study of pDC biology and understanding the nature of pDCs associated with several neoplasms could pave the way for new therapeutic possibilities [11].

3.3 Circulating endothelial cells and endothelial progenitors: Circulating endothelial cells (CECs) have been recently indicated as potential biomarkers of angiogenesis in several types of cancer. Previous experiences have demonstrated that they can be affected by both disease status and anti-cancer treatment. CECs are surrogate markers of endothelial damage [12]. Increased CEC counts are observed in cancer patients, where they seem to be promising biomarkers for tumor progression and monitoring therapeutic effects [13,14].

Endothelial progenitor cells (EPCs) are a blood-circulating cell population that is able to form endothelial colonies *in vitro* and to promote vasculogenesis [7]. Absolute baseline number and changes in number and viability of CECs (and CEPs) have shown predictive value for response to an antiangiogenetic therapy for breast cancer [15]. High CEC levels at baseline were reported in 2 studies using flow cytometry to indicate a better outcome in breast cancer. A low baseline absolute number of CECs (defined on the basis of different cutoffs in each study) seems to be a predictor of better PFS in metastatic colorectal cancer patients (mCRC)[16,17,18].In mCRC, conflicting data also emerged regarding baseline CEC counts and other outcomes (ie, ORR and OS). Hence an unequivocal conclusion cannot be reached on the prognostic or predictive value of the kinetic modification of CECs during treatment, as each study evaluated different time points. Furthermore, chemotherapy treatments performed in association with bevacizumab (Anti-VEGF) were not uniform. Major limitations of quantifying CECs and CEPs by FCM include: i) Multiple flow cytometric protocols and methods; ii) heterogeneity in sample processing phases; iii) absence of consensus on standardized and validated MoAb panels for CECs and EPCs; iv) specific compensation and gating strategies; v) application of specific data analysis programs. The lack of a consensus on a valid CEC and EPC phenotypic definition and the multitude of the used flow cytometric methods have resulted in a great heterogeneity in the reported blood levels of CECs and EPCs, which limits the impact of the obtained data in clinical practice. Despite limitations, CECs and their subpopulations remain promising predictive biomarkers in patients treated by antiangiogenic therapy. Reaching a consensus on phenotypic characterization will be crucial to critically evaluate the role of these cells in comparable, well-designed, biomarker-based clinical trials for the early identification of patients who can be reasonably excluded from treatment, as well as to develop therapeutic approaches able to overcome acquired resistance to anti-VEGF strategies [19].

3.4 Cancer Stem cells: According to the cancer stem cell hypothesis, a tumor consists of more differentiated highly proliferating cells, which constitute the tumor bulk and sustain tumor growth, and undifferentiated slow-cycling cells with self-renewing capacity, the so-called cancer stem cells (CSCs) [20,21,22]. CSCs reside in special niches and have the potential to reconstitute a tumor after an otherwise successful therapy and may be responsible for tumor metastasis. There is a need to establish reliable methods to identify and isolate CSCs for their better understanding and also to develop effective treatment strategies. Both identification of cluster of differentiation of surface antigens and functional properties characteristic of CSCs have been utilized. The expression of distinct markers varies among different tumors and may include singly or in combination antigens like CD133, CD44, CD24, CD176, alpha-2-beta-1 integrin. However, researchers have described ‘phenotypic instability’ in CSCs which means that the cellular phenotype of stem cells can vary or change during the development and growth of tumors in vivo and under different conditions in cell culture [23].

Side population analysis is based on the stem cell-specific activity of certain ATP-binding cassette (ABC) transporter proteins, which are able to transport fluorescent dyes out of the cells. The high expression of ABC transporter proteins in CSCs enables a flow cytometric identification of these cells as a ‘‘side population’’ (SP). In flow cytometric analysis, SP cells usually form a distinct small cell population (typically<0.1%) showing little or no fluorescence with vital DNA dyes like Hoechst 33342, SYTO13 or Rhodamine 123. Vital dyes are effectively eliminated from ABC transporter protein expressing cells [24]. Another functional property of stem cells which can be utilized is the stem cell-specific presence of aldehyde dehydrogenase
isoform 1 (ALDH1) which can be used for CSC labeling. As a single marker ALDH1 has limitations since its activity can be altered by chemotherapeutic treatments however it can be combined with other more stable CSC-specific detection methods like cell surface marker expression analysis and/or SP analysis. However, the flow cytometric analysis of these CSC features requires specific technical adjustments.

3.5 Tumor Infiltrating Immune cells: Earlier presumed to be contaminants in solid tumor flow cytometry, it is well-established now that tumor-infiltrating immune cells play a very important role in tumor development and control. Depending on the type of cells and their functional interactions, immune cells may play a key role in suppressing the tumor or in providing support for tumor growth. Efforts for identification and characterization of immune cells present within the tumor for understanding their effects on tumor behavior and to find new therapeutic targets are being made. It has been revealed in various studies that CNS tumors show variable infiltration by tumor associated macrophages/ microglial cells (TAM) and lymphocytes (particularly T-cells and NK cells, and less frequently also B-cells). TAM infiltrate gliomas where they frequently show an immune suppressive phenotype and functional behavior in contrast, infiltration by TAM may be very pronounced in meningiomas, where the immune infiltrates also contain greater numbers of cytotoxic T and NK-cells associated with an enhanced anti-tumoral immune response. TAM and Tumor associated neutrophils (TANs) are recruited by Pancreatic ductal adenocarcinomas and facilitate immune evasion. Targeting of CCR2+ TAM and CXCR2+ TAN improves antitumour immunity and chemotherapeutic response in PDAC [25]. FCM has been used other than immunohistochemistry and immunofluorescence in multiple studies for identification of immune cells in tumors and in cell lines. It has the advantage of identification and characterization of heterogeneous cell populations coexisting in tumor samples[26,27].

Technical considerations for solid tumor FCM

FCM applications of solid tumor are technically more challenging as the most important prequisite for FCM is ‘cells in suspension’. Source of tumor may include fresh surgical specimens, FNAs or frozen/paraffin embedded tissues. Sample disaggregation can be achieved by mechanical or enzymatic techniques or a combination of both. The idea is to recover large majority of cells which should be intact in a short period of time. Fixation and permeabilization are required for intracellular antigens. For many experiments Live/ dead discrimination would be required as the cell suspension from solid tumors contain large amounts of debris with red cells, fibrous stroma and cell fragments. Given the wide-ranging scope of solid tumour flow cytometry, and the relative difficulty of sample procurement, it is convenient to cryopreserve fixed cell suspensions for subsequent use.This can be done by placing aliquots containing typically 10^6 cells in Eppendorf tubes, gently spinning down, and then resuspending in 0.5 ml of freezing mixture consisting of 20% glycerol and 10% fetal calf serum in tissue culture medium. Most intracellular proteins, as well as light scatter and DNA content, are preserved for up to several months when these samples are frozen at -20 or below [28].

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Basics of Flow Cytometry

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To drive an automobile one does not need to know how to design an alternator but should understand if it's operating correctly and optimally. Likewise with a flow cytometer or cell sorter; to properly operate a cytometer and interpret data, one must understand its basic components and their operation in order to obtain valid and optimized data. The goal of this lecture is to familiarize those new to the field of flow cytometry with its basic components and their correct usage. The key components including fluidics, lasers, optics, electronic detectors, analog to digital converters and pulse processors will be described in sufficient detail to give the new operator/user a basic understanding.

Cell Cycle Analysis by Flow Cytometry

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Crissman and Steinkamp (1973) published a paper on use of propidium iodide for flow cytometric analysis of DNA in cells fixed with ethanol and digested with RNase. Subsequently, Krishan (1975) reported that propidium iodide solutions made in hypotonic sodium citrate could lyse the cells and directly stain the cells for DNA content and cell cycle analysis. The method described below is rapid, uses small amount of material and has been extensively quoted and used for flow cytometric analysis of DNA aneuploidy and cell cycle distribution.

Crissman HA, Steinkamp JA. Rapid, simultaneous measurement of DNA, protein,and cell volume in single cells from large mammalian cell populations. **J Cell Biol**. 1973 59:766-71.

Krishan A. Rapid flow cytofluorometric analysis of mammalian cell cycle by propidium iodide staining. **J.Cell Biol.** 1975 66:188-193.

Abstracts

Oral Presentations
Oral Paper Basic Science

OPBS-1

TITLE: Mesenchymal cell derived cellular products and their regenerative properties

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Objective: Intracellular communication has shown lot of importance in various clinical conditions in repair of injured cells. There has been burgeoning interest in intercellular organelle and products transfer and bio-energizing the repair and regeneration. Studies have demonstrated that cellular product derived from human liver stem cells promoted hepatocyte proliferation and suppressed hepatocyte cell death. Liver cirrhosis is irreversible and mostly ends up with complete loss of architecture. Exosomes are endogenous nanoparticles that play significant roles in mediating the biologically active cargo, such as nucleic acids, proteins and in intercellular communication. Intracellular communication has lot of importance in repair of injured cells. The liver is an ideal target for exosome therapy due to the intrinsic regenerative capacity of hepatic tissue. The aim of this study is to investigate the effect of mesenchymal cell derived exosomes in an established carbon tetrachloride (CCl4)-induced liver injury mouse model.

Methods: Mesenchymal cells were used to introduce and characterize the exosomes. Characterization of exosome was done using specific markers (CD81, CD63) through flowcytometry. The protein content of the intense exosomes was determined using a BCA protein assay kit. Exosomes were recognized by transmission electron microscopy and other markers. Exosomes were administrated into CCl4 induced mouse model. Efficacy of exosome therapy was determined by biochemical and histopathological assessment.

Results: Study has revealed that transplantation of exosomes derived from ascitis reduced the external fibrous capsules and got their textures soft. Hepatic inflammation was also reduced and collagen deposition in CCl4 induced fibrotic liver. Exosomes also significantly recovered serum liver function test activity, decreased collagen levels, transforming growth factor (TGF)-b1 and phosphorylation Smad2 expression in vivo.

Conclusions: These results suggest that exosomes could ameliorate CCl4-induced liver injury by protecting hepatocytes. This provides a novel approach for the treatment of liver injury.

OPBS-2

TITLE - Cellular immune profile of infants with neonatal cholestasis and infected with human cytomegalovirus

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Objective: Neonatal Cholestasis (NC) is failure of bilirubin secretion resulting in conjugated hyperbilirubinemia and jaundice. In India, NC constitutes 19% to 33% of all chronic liver diseases in children and has been associated to congenital or acquired viral infections. Human Cytomegalovirus (HCMV) has been proposed as a possible etiological agent of NC. HCMV infection is a common perinatal infection with 2% of infants affected at birth. In our earlier report Goel et al. 2018, HCMV was detectable by PCR in the liver tissues of more than half the infants with NC and had a positive serology for HCMV IgM, where presence of IgM indicates recent infection. To further investigate the role of HCMV pathogenesis in this context we analysed cellular immune subsets in these infants with NC and HCMV infection.

Materials and Methods: In this study, we compared cellular immune subsets between two groups: 1) HCMV infected IgM Negative: n=21 and 2) HCMV infected IgM Positive: n=18. Immunophenotyping of T cell subsets: CD4+ Tcells, CD8+ Tcells, CD4 and CD8 naïve memory and effector memory, and Treg cells were carried out by surface staining of whole blood with anti-CD3, anti-CD4, anti-CD25 and anti-CD127 antibodies. Immunophenotyping of NK cells were carried out by surface staining with anti-CD3, anti-CD4 and anti-CD16 antibodies and intracellular staining using anti-Granzyme-B antibody. Flow cytometric acquisition and analysis were performed on a BD ACCURI C6 flow cytometer.

Results: There was no significant difference in the frequency of Treg cells and total T cells between the two groups, however we found higher frequency of CD8+ T cells and lower frequency of CD4+ T cells in the HCMV infected IgM positive group. Also, this group has higher frequency of CD8+ effector memory cells. Increase in the CD8 effector memory was mirrored in the cytotoxic potential of T cells where we see an increase in Granzyme-B positivity in both CD4+ and CD8+ T cell population in HCMV infected IgM positive group. There was no significant difference in either total NK frequency or CD16 high and CD16 low frequency and in NKT frequency, however as seen in case of total T cells, when we looked at the Granzyme-B positive fraction of NK cells and NKT cells we notice an increase in the Granzyme-B positivity in both NK cells and NKT cells in HCMV infected IgM positive group.

Conclusion: Increase in cytotoxic potential in both T cell subsets and the NK subsets suggests that this increase is possibly driven by acute viral infection and associated inflammation in these infants who are HCMV infected IgM positive. This could be an immune signature that is associated with acute or recent infection and might have implications for pathology of neonatal cholestasis.

OPBS-3

TITLE: Cellular HIV reservoirs and efforts towards purging the latent virus

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Objective: Anti -retroviral therapy (ART) has shown to be efficient in suppressing viremia but it cannoteliminate HIV from latent cellular reservoirs containing integrated HIV DNA. These cells are not recognised by the immune system due to minimum HIV production. Hence, it is necessary to estimate the distribution of proviral burden in circulating cellular subsets such as CD4+ T cells and monocytes. Also, it is crucial to reactivate HIV from its cellular reservoirs to eliminate reservoirs. Therefore, we aimed to test the efficiency of some latency reversing agents (LRAs) on J-lat clone (latently infected cell line) as well as 1° cells.

Methods: J-lat (10.6) cells harbour single copy of integrated HIV (*nef* replaced by GFP). J-lat cells (2.5 x 10^6 cells/well) were incubated with/without LRA for 48 hrs in 96 well plate. GFP positivity was measured at 24 hr interval.PBMCs were isolated and infected *in-vitro* with JR-CSF (HIV-1 strain) for 4hrs at 37°C. Cells were washed and cultured. 3 days post infection CD4+ T cells subsets (naïve, central memory, transition memory, effectors) were sorted using FACS Aria III based on CD3, CD4, CD28, HLA-DR, CCR7, CD45RA expression. Sorted subsets were incubated with/ without LRA and cultured for 4 days. HIV production was measured by p24 ELISA assay using culture supernatant. DNA was isolated from the sorted cellular subsets to estimate integrated HIV DNA (Alu-gag) as well as total HIV DNA (*env*) by nested PCR.

Result: Treatment of J-lat cell line with PHA increased GFP positivity by 6% after 24 hrs and further increased by 1% after 48 hrs.We have sorted CD4+ T cell subsets with more than 95% purity and detected integrated HIV copies in sorted cells using Alu-gag PCR. Extracellular p24 was below detection limit in all the sorted cells even after stimulation.

Conclusion: We have successfully induced GFP production in J-lat cells indicating reactivation of latency.We have optimised the Alu-gag PCR to detect integrated HIV copies. We have been successful in sorting CD4 T cell subsets with more than 95% purity.We further aim to test these preliminary studies in the sorted cellular subsets (CD4 T cells, monocytes) of primary samples. In future experiments we will try to increase the yield of sorted cells to get p24

OPBS-4

***TITLE: HIF-2α* stabilization in platelets induces prothrombotic state through *PAI-1* synthesis and extracellular vesicles release**

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Objective: Oxygen-compromised environments like high altitude are associated with platelet hyperactivity. Platelets confined within the relatively impervious core of thrombus have restricted access to oxygen, yet they continue to perform energy-intensive procoagulant activities that sustain the thrombus. Therefore, studying platelet signaling under hypoxic stress is critical to our understanding of the mechanistic basis of thrombus propagation/sustenance. We aimed to elucidate the adaptive signaling in platelets in response to hypoxia and determine its potential role in mediating thrombogenesis.

Materials and methods: Platelets were isolated by differential centrifugation from fresh human blood. Hypoxia simulation was induced using hypoxia chamber. Protein expression analysis was carried out by immunoblotting. Extracellular vesicles (EVs) were quantitated by nanoparticle tracking analysis (NTA). Intracellular calcium was measured by fluorescence spectrophotometry using Fura-2 AM. PAC-1, P-selectin and annexin binding was analysed by flow cytometry. Imaging of arteriolar thrombosis by Intravital microscopy. Standard statistical methods were used and test were considered significant at $p<0.05$. Data was analysed by using GraphPad Prism 7 software.

Results: We report here that, hypoxia-inducible factor (HIF)-2α is translated from pre-existing mRNAs and stabilized against proteolytic degradation in enucleate platelets exposed to hypoxia. Exposure of platelets either to hypoxia or hypoxia-mimetics (DMOG and DFO) significantly upregulated synthesis of plasminogen-activator inhibitor (PAI)-1 and shedding of EVs, both of which potentially contribute to pro-thrombotic phenotype associated with hypoxia. Platelets exposed to hypoxia-mimetics, too, evoked significant rise in intracellular calcium. Whereas no significant changes were observed in PAC-1, P-selectin and annexin binding in platelets exposed to hypoxic stress. Administering hypoxia-mimetics into mice accelerates thrombus formation in mesenteric arterioles. In agreement, platelets from COPD patients and high altitude residents exhibiting thrombogenic attributes have abundant expression of HIF-2α and PAI-1.

Conclusion: Hypoxia signaling in platelets contributes to a prothrombotic state. Thus, targeting the same could be an effective anti-thrombotic strategy.

OPBS-5

TITLE: Development of novel and efficient method for in vitro germ cell differentiation from embryonic stem cells using flow-cyometric techniques.

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Primordial germ cells (PGC) are precursors of germ cells arise at post implantation stage of embryo. PGC differentiate from a subset of the cells of the epiblast stem cells (EpiSC) in response of signaling molecule from the vicinity cells. These PGCs further differentiate into gonocyte and then sperm or oocyte. Recently different regimes for the in vitro germ cell differentiation from ESCs and induce pluripotent cells (iPSCs) have been identified but these protocols are inefficient and very complex. These In vitro derived germ cells have potential to differentiate in to both male and female gametes and have great potential to rescue the infertility.

In the study we created a double reporter ESC line for two different stage germ cell markers, DPPA3 and DDX4. DPPA3 expresses exclusively in primordial germ cells. DDX4 is also a germ cell specific marker express into pre meiotic germ cells. We tagged DPPA3 with mCherry and DDX4 with GFP, endogenously using CRISPR-CAS9 mediated Knock-In. Using this cell line we develop an efficient and simplest protocol to differentiate ESC to PGC and further into spermatogonia. We achieve up to 50% cells which differentiate into primordial germ cells and these cells are capable to further differentiate into spermatogonia. By using the developed protocol we demonstrated the role of a novel gene Tex13 which we identified as a target for male infertility. The study will be helpful to explore the regulatory mechanisms for germ cell development. The protocol will be also useful for developing regenerative medicines to rescue the infertility. To develop the protocol we use flow cytometry as a major tool. To create cell line we used FACS (Moflow, Beckman Coulter) for purification of positive cells. To analyses the efficiency of the germ cell differentiation we used flow cytometer (BD LSRFortessa). We achieved most efficient regime for germ cell differentiation.

Oral Paper Clinical Science

OPCS-1

TITLE: Flow Cytometry Based Diagnosis of Leucocyte Adhesion Defect, Autoimmune Lymphoproliferative Syndrome And Chronic Granulomatous Disease

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Objectives: Primary immunodeficiency (PID) is a broad disease spectrum comprising more than 350 distinct disorders with distinct and overlapping clinical and laboratory features. Flow cytometry plays an important role both for screening and diagnosis of various PID. Here we describe our experience with flow cytometry based diagnosis of Leucocyte Adhesion Defect (LAD), Autoimmune Lymphoproliferative Syndrome (ALPS) and Chronic Granulomatous Disease (CGD).

Materials and Methods: This study includes 421 samples analysed for LAD (n=48), ALPS (n=270) and CGD (n=103). Flow cytometry acquisition were done with BD FACSCanto and BC Navios 10 colour flow cytometers. The following antibody-flourochrome conjugate panels were used: CD18-FITC, CD11a-FITC, CD11b-APC, CD11c-PE, CD19-PerCP-Cy5.5 and CD56-APC for LAD, CD3-APC, TCRαβ-FITC, CD4-PE, CD8-PE, CD45-PerCP for ALPS while Phorbolmyristate acetate (PMA) and Dihydrorhodamine (DHR) were used for stimulation and cell dependant oxidation in CGD

Results: Among the 48 patients evaluated for LAD, 18 patients showed complete loss of CD11a, CD11b, CD11c and CD18 expression in the neutrophils. The median (range) percentage of neutrophils expressing CD11a, CD11b, CD11c and CD18 were 1.2%(0%-5.6%), 0.85%(0.1%-6.1%), 0.2%(0%-3.1%) and 0.1%(0.1%-15.7%) respectively while the expression were normal in LAD negative patients (CD11a-98.2%, CD11b-99.3%, CD11c-99.2% and CD18-99.7%). Among the 270 patients evaluated for ALPS, 53 patients (19.6%) had an increase in CD3+TCRαβ+CD4-CD8- (Double negative T cells) consistent with a diagnosis of ALPS. The median percentage of double negative T cells among total lymphocytes and CD3+ T cells were 2.38%(1.6%-26.2%) and 3.31%(2.38%-51.1%) respectively. CGD is defined by the lack of increase in the DHR mean fluorescence intensity (MFI) following stimulation of neutrophils with PMA and was seen in 18 (median patient/control MFI:0.485 vs 1.002;$p<0.001$) among the 103 patients [Median MFI post PMA stimulation: CGD-432.5 (202-686), controls-902.5 (760-955)].

Conclusion: While genetic analysis is required for the definitive diagnoses of PID, flow cytometry provides a rapid cost-effective tool to effectively diagnose ALPS, CGD and LAD.

OPCS-2

TITLE: Standardization of Flow Cytometric staining of Bcl-2, Bcl-6 And Mum-1 Expression In Non-CLL B-Cell Non-Hodgkin's Lymphoma

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Institution: Tata Memorial Hospital, Mumbai

Objective: The expression of nuclear transcriptional proteins such as BCL-2, BCL-6 and MUM-1 are essentially used for the diagnosis and classification of non-Hodgkins lymphoma (NHL) using immunohistochemistry. Flow cytometry (FC) is extensively used in the diagnosis and classification of hematological neoplasms using a variety of surface, cytoplasmic and nuclear proteins. However, flow cytometric staining of these markers is not standardized for clinical utility. We have standardized the FC-protocol for the staining of BCL-2, BCL-6 and MUM-1 and evaluated their expression pattern in B-NHL.

Methods: We studied FC-staining of BCL2 (PE, clone-REA872), BCL6 (APC, clone-REA373) and MUM1 (APC, clone-REA201FC) in peripheral blood (PB), bone marrow (BM), lymph node (LN) and FNAC samples from non-CLL B-cell NHL. We evaluated four different kits i.e. Fix-and-Perm (Invitrogen), FACS-lyse (BD), Foxp3 Transcription Factor Staining Buffer Set (eBioscience), and True-Nuclear Transcription Factor Buffer Set (Biolegend) for fixation and permeabilization of these 44markers. FC was performed on Cytoflex (Beckman-Coulter) flow-cytometer and data-analysis was performed using Kaluza-v2.1-software.

Results: Of the four fixation and permeabilization kits, Foxp3 Transcription Factor Staining Buffer Set (eBioscience) has given the best results for BCL-2 and BCL-6 staining and FACS-lyse was found best for MUM-1 staining with 30 minutes of incubation. We studied 47 samples (PB-7, BM-34 and FNAC-6) submitted for the diagnosis of NHL. BCL-2 and BCL-6 was studied in all 47 samples and MUM-1 was studied in 18 samples. The results of expression of all these three markers are given in the Table-1 (given below on the next page).

Conclusion: We have standardized the flow cytometric protocol for analysis of BCL-2, BCL-6 and MUM-1 expression in B-cell NHL. This protocol allows rapid diagnosis and classification of B-cell NHL by flow cytometry and limits the need for confirmation of diagnosis on immunohistochemistry on biopsy samples.

OPCS-3

TITLE - Clinical Relevance of The Proportion Of Residual Polyclonal Plasma Cells And Total Plasma Cells In The Diagnostic Bone Marrow Of Newly Diagnosed Multiple Myeloma Patients Managed Without Autologous Stem Cell Transplantation

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Institution: Tata Memorial hospital, Mumbai

Objective: Studies have demonstrated the value of residual polyclonal PC (pPC), and total PC (tPC) in the prognostication of MM treated with autologous stem cell transplant (ASCT) using flow cytometric immunophenotyping (FCI). However, it has not been studied in patients who did not undergo ASCT. Herein, we present a study highlighting the prognostic relevance of the proportion of residual pPC and tPC in bone marrow aspirate (BM) on FCI in MM patients managed without ASCT due to limited resources.

Methods: We prospectively studied the proportion of residual pPC in tPC and tPC in all viable cells in BM samples from newly diagnosed MM patients treated with VCD-protocol using 8-10 color FCI.

Results: We studied FCI in BM and progression-free survival (PFS) in 123 patients of MM (age: median-59 years, range- 34 to 78 years and M: F-1.8. Median-values (range) of M-protein levels were 3.9 g/dl (0.05-12 g/dl). The patients were ISS stage I - 57/119 (47.9%), ISS-II - 35/119 (29.41%) and ISS-III - 27/119 (22.69%). Median (range) of follow-up of MM-patients was 19.1 (4.7-51.5) months. On Kaplan-Meier survival analysis, the median PFS in patients with <10% pPC was 22.5 months, while the median PFS in patients with ≥10% pPC was 48.4 months (P= 0.0004). Similarly, the median progression-free survival (PFS) in patients with <2.5% tPC was 41.1 months, while the median PFS in patients with ≥2.5% tPCs was 22.9 months (P= 0.004). On Cox regression analysis, the hazard ratio for <10% pPC and >2.2% tPC were 4.24, 2.05 respectively.

Conclusion: Our results demonstrated the clinical relevance of detecting residual polyclonal (normal) PCs and total PC in MM-patients at diagnosis using FCI. It provides a category of good prognostic MM with >10% pPC and <2.5% tPC that may not require ASCT in resource-limited settings.

OPCS-4

TITLE: **The Frequency, Cytogenetic Spectrum and Short-Term Treatment Response Of CRLF2 Positive B-Lineage Acute Lymphoblastic Leukemia**

Diksha Dev Yadav[1], Sreejesh Sreedharanunni[1], Sonia Rana[1], Minakshi Gupta[1], Parveen Bose[1], Alka Khadwal[2],Man Updesh Singh Sachdeva[1], Pankaj Malhotra[2], Amita Trehan[3], Neelam Varma[1].

Institution: Departments of Hematology[1], Adult clinical Hematology unit of Internal Medicine[2], Pediatric Hematology/Oncology unit[3], Postgraduate Institute of Medical education and Research, Chandigarh – 160012

Objectives: To study the frequency of expression of CRLF2 by flow-cytometry (FCM) in B-acute lymphoblastic leukemia (B-ALL); their association with recurrent cytogenetic abnormalities; and to correlate with post-induction measurable residual disease (MRD).

Materials and methods: In patients diagnosed of B-ALL, the expression of CRLF2 was determined on blasts by fluorescence minus one method using phycoerythrin or PerCP-eFluor710, conjugated anti-TSLP receptor antibodies (clone EBIO1A6, eBioscience). The laboratory data and the results of Fluorescence in-situ hybridization (FISH) /RT-PCR data for *ETV6-RUNX1, KMT2A, BCR-ABL1 and TCF3-PBX1* translocations was documented. The patients were followed up for post-induction MRD by FCM.

Results: During 10 months study period, a total of 209 cases underwent testing for CRLF2 by FCM. The age of the patients ranged from 7 months to 61 years [median 11 years; pediatric (52%), adults (48%)] with a male: female ratio of 1.75:1. 60.2% of patients (n=126) were in NCI high-risk group. CRLF2 expression (>10%) was detected in 9.6% (n=20) of patients. The percentage of CRLF2+ve blasts ranged from 11.7%-99.3% (Median 17.4%). There was no significant difference in CRLF2 expression between NCI high risk and standard risk (p=0.61) or between adults and paediatric subgroups (p=0.84). RT-PCR/FISH data as mentioned above was available 19 patients with CRLF2+vity. *BCR-ABL1* and *iAMP21* was positive in 4 (21%) and 2 (10.5%) patients respectively. Among the cases negative for *BCR-ABL1* translocation (n=15), FISH testing revealed CRLF2 translocation in 5 cases. Post-induction MRD testing was performed in 108 cases (51.6%) with MRD being positive in 34 cases (31.5%). There was no significant difference (p=0.94) in the frequency of MRD positivity between CRLF2+ve (4/12; 30%) and CRLF2-ve subgroups (31/96; 32.3%).

Conclusions: CRLF2 overexpression is seen in 9.5% of B ALL and is associated with *CRLF2* (26%), *BCR-ABL1* (21%) translocation or *iamp21* (10.5%) in >50% of the cases. MRD positivity is not significantly higher in CRLF2+ group in our study**.**

OPCS-5

TITLE: Screening of Primary Immunodeficiency Disorders – A Cost Effective Panel Based Approach Saves Time And Effort

Ananthvikas J[1], Pradeep Kumar V[2], Kavya Shankaranarayana[3], Sagar Bhattad[4], Sujay Prasad[5]
Institution: Neuberg Anand Reference Laboratory[1,2,3,5], and Aster CMI Hospital[4], Bangalore

Objectives – According to recent estimates, 1 in 1200 children are affected by some form of primary immunodeficiency disorder (PID). Early diagnosis and specific therapy greatly improve the chances for cure. We aimed to evaluate the utility of a cost-effective test-panel based approach in the screening of common PIDs. Materials and methods – A PID screening panel designed in December 2018 comprised T-B-NK lymphocyte subset flow cytometry, Dihydrorhodamine and Nitroblue tetrazolium tests and Serum immunoglobulin levels. 154 cases of suspected PID were analyzed using this panel in a 7-month period (January – July 2019) and results correlated with clinical symptoms. Confirmatory tests were then performed in select cases.

Results – Patients ranged in age from neonates to 37 years (68% under 4 years age) with a Male: Female ratio of 1.7:1. 104 of the 154 screened samples showed normal results. The screening tests were suggestive of the following diagnoses (number of cases in parenthesis): chronic granulomatous disease (9), Hyper-IgE syndrome (9), Hypogammaglobulinemia (6), SCID (7), Hyper-IgM syndrome (8), X-linked agammaglobulinemia (3), CVID (2), CD4 cytopenia (2), Selective IGA deficiency(1) and Myeloperoxidase deficiency with Leucocyte adhesion defect-1 (1). Following the results of the screening panel, a subset of cases were further confirmed on further testing: whole exome sequencing [CGD(5), Griscelli syndrome(1), LAD1(1), Hyper-IgE syndrome(3), Hyper-IgM syndrome(3), SCID(4), XLA-(1)], Naïve T cell subsets [SCID(1), CD4 cytopenia(1)], HIV serology (1) and CD18 flow cytometry (1). On follow-up, atleast 7 of these children have received bone marrow transplants (SCID – 4, CGD – 1, HyperIgM – 1, LAD – 1).

Conclusion –A panel-based approach as described here helps save effort and precious time and is cost-effective enough to help screen a larger population. Early identification of a PID and initiation of specific therapy can greatly improve the outcome of these cases.

Interesting Case presentations

Non MRD category

S No.	Name	Affiliation	Cases summary
1	A. Arun Kumar	Hematology, CMC, Vellore	2/M with abdominal distension
2	Jayasudha AV	Pathology, RCC, Trivandrum	Hepatosplenic T-cell Lymphoma
3	Simi CM	Patholgy, RCC, Trivandrum	Two cases (61/M with LAP and organomegaly, 53/M with LAP)
4	Ananthvikas J	Neuberg Anand Reference Laboratory, Bangalore	1/F with repeated infections
5	Avinash Gupta	HBCH, Varanasi	A rare case of FGFR1 rearrangement with both aberrant B and T lymphoid blast in the marrow
6	Amrita Saraf	Hematology, Gangaram Hospital, New Delhi	63/M with persistent lymphocytosis
7	Vineeta Paswan	SGPGI, Lucknow	To be or not to be: an unusual case of ETP-ALL

MRD Category

S No.	Name	Affiliation	Cases summary
1	Kunal Sehgal	Sehgal Path Lab, Mumbai	A B-ALL post completion of MRD therapy deveops a secondary AML
2	Karthik Bommannan B.K	Oncopathology, Adyar Cancer Institute	Unusual pattern of immunoglobulin light chain expression in plasma cells evaluated during minimal residual disease assessment.
3	Devasis Panda	TMH, Mumbai	Utility of single tube 16c antibody panel for detection of minimal residual disease of unusual immunophenotype in a case of acute myeloid leukemia in a 4 year old male
4	Mousumi Kar	TMH, Mumbai	Minimal residual disease assessment in a case of lymphoplasmacytic lymphoma in a 27year old male

Poster Presentation
Poster Presentation – Basic Science (PBS)

PPBS-1

TITLE - MAPK signaling alteration involving hematopoietic dysregulation in experimental myelodysplastic syndrome with an impact on hematopathology

Suchismita Daw, Sujata Law*
Institution: Dept of Biochemistry and Medical Biotechnology. Calcutta School of Tropical Medicine.

Objective: Hematological disorders like myelodysplastic syndrome(MDS) arises due to clonally dysregulated hematopoiesis forming dysplastic cells which lacks proper maturity and immune functional capacity. Myelodysplastic syndrome is considered globally as heterogenous group of neoplasm which often proclaims leukemic progression. The heterogeneity is reflected not only in clinical manifestations of the disease but also in salient causes of disease development. In spite of multiple therapeutic modalities, shortfall towards treatment of this disorder still persists. The focal point of tussle suggested toward defects, which are not confined to any unifying cellular signalling. The pathobiology of the disease often experiences an intriguing paradox involving '*hyperproliferative bone marrow with pancytopenic peripheral blood'*. The focussing zone was MAPK signaling in the hematopoietic stem progenitor compartmental (HSPC) dysregulation during the course of alkylator(ENU) induced myelodysplasia.

Methods: Assessment of cell morphology of peripheral blood and bone marrow as well as cytochemistry, histochemistry, karyotyping and flowcytometric analysis were taken into consideration.

Results: The phospho-protein status of Receptor Tyrosine Kinases (RTK's) like FLT3(MDS: 15.38±0.40,Control:11.61±0.50, p=0.0005), PDGFR(MDS:48.90±1.0, Control:26.46±0.50, p<0.0001) EGFR(MDS:51.40±0.43,Control:48.40±0.60,p =0.0008) were markedly increased that activated MAPK signaling proteins[Ras(MDS:45.28±0.72,Control:19.35±0.45,p<0.0001),phospho-Raf(MDS:13.38±0.62, Control:8.61±0.39, p=0.0003), phospho-MEK(MDS:23.71±0.52,Control: 15.07±0.90, p<0.0001) and phospho-ERK(MDS:47.52±0.48,Control:34.31±0.69, p<0.0001)] which finally executed their tasks by transcription of c-Myc(MDS:52.89±0.80,Control:46.08±0.10, p=0.0001) and Rb(MDS:45.35±0.65, Control:32.33±0.67,p<0.0001) proteins leading to uncontrolled cellular proliferation of the HSPC's. Activated c-Jun(MDS:51.74±0.26,Control:38.62±0.40, p<0.0001) revealed stress related apoptosis and the phenotypic expression of hematopoietic stem cell marker CD 150(MDS:15.04±0.94,Control: 9.28±0.52, p=0.0007) and progenitor cell marker CD 90(MDS:56.98±0.73,Control: 102.53±25, p<0.0001) also established a mechanistic correlation with MAPK signaling .

Conclusion: Altogether, the role of activated MAPK signaling may have led to hyperproliferation and concurrent enhanced apoptosis of abnormal HSPC's which gradually headed towards premalignant transformations during the disease.

PPBS-2

TITLE - Interaction of Fibrinogen With Integrin $\alpha_{IIb}\beta_3$ Expressed On Platelet-Derived Extracellular Vesicles

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Objectives: Here we studied interaction of integrins αIIbβ3 expressed on platelet-derived extracellular vesicles (PEVs) with fibrinogen by flow cytometry and role of fibrinogen in PEV generation by nanoparticle tracking analysis (NTA).

Materials and methods: Platelets were isolated from fresh human blood by differential centrifugation. Platelets were induced to generate PEVs in presence of calcium ionophore (A23187), followed by centrifugation to pellet platelets. Supernatant containing PEVs was collected for quantitation by nanoparticle tracking analysis (NTA). Binding of Alexa Fluor 488-labelled fibrinogen on integrin αIIbβ3 expressed on PEVs was analysed by 4-quadrant logarithmic amplification using CellQuest Pro software.

Results: The result suggests that pre-incubation of platelets with RGDS significantly attenuated (by 39.28%, $p < 0.05$) EV release when stimulated with calcium ionophore. PEV integrins αIIbβ3 interact with fibrinogen in an RGD independent manner reflecting that amino acid sequence 109-171 on β3 integrin is not exposed to bind with fibrinogen in case of PEVs.

Conclusion: This study is based on characterization of PEVs released from platelets. Our observations are reflective of critical differences in αIIbβ3 conformer expressed on extracellular vesicles from that on platelet surface. PEVs express active conformer of integrin αIIbβ3 on the outer membrane surface, which has strong affinity for fibrinogen revealing their potential role in homeostasis and thrombosis.

PPBS-3

TITLE: Pro inflammatory role of self DNA-ll37 complexes in the pathogenesis of type-1 diabetes: how dying beta cells contribute towards their own destruction.

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Institution: Departments of Pediatrics[1] and Endocrinology [2]. Post Graduate Institute of Medical Education and Research (PGIMER), Chandigarh, India.

Objectives: Initial stages of type 1 diabetes (T1D) involve infiltration of plasmacytoid dendritic cells (pDCs) and monocytes in pancreatic islets. Our Objective was to study whether DNA-LL37 complexes play a role in the activation of pDCs and monocytes towards a proinflammatory phenotype during pathogenesis of type 1 diabetes.

Materials and methods: We recruited recently diagnosed 40 T1D and 15 healthy control (HC) subjects to study the effects of DNA-LL37 complexes on pDCs and monocytes in terms of induction of IFN-α, enhancement in antigen presenting capacity and activation of CD4+ T cells.

Results: LL37 formed stable complexes with genomic DNA, conferring protection from DNase degradation and DNA-LL37 complexes were internalized by pDCs and monocytes via endocytosis. Following in-vitro stimulation with DNA-LL37 complex, we observed increase in the frequency of IFN-α expressing monocytes and pDCs, with T1D group demonstrating a higher frequency of IFN-α+ pDCs, both prior to and after stimulation. Post-stimulation, the frequency of pDCs and monocytes expressing CD80 and CD86 increased with pDCs gaining mature phenotype with reduced ILT7 expression. Further comparison revealed higher frequency of activated pDCs, and monocytes in the T1D group. Next, we found an increase in the frequency of T cells expressing CD69 when co-cultured with stimulated pDCs and, T cells expressing CD69 and CD71 when co-cultured with stimulated monocytes in the T1D group. Finally, in vitro co-cultures demonstrated increased beta cell apoptosis in the presence of stimulated pDCs and monocytes.

Additionally, we assessed tolerogenic Indoleamine 2,3-dioxygenase (IDO) expression in pDCs and monocytes and TREX1 in monocytes. We observed reduced expression of IDO and TREX1 in monocytes of T1D subjects, which supports the notion that monocytes in T1D are defective and skewed towards pro-inflammation.

Conclusion: Our results suggest that DNA-LL37 complexes activate pDCs and monocytes towards a proinflammatory phenotype during early pathogenesis of T1D.

PPBS-4

TITLE - Trolox inhibits caspase-mediated apoptosis in high glucose exposed rat cardiomyoblasts by restoring cellular redox state

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Objective: Diabetes has emerged as a major global health issue in the 21st century and individuals suffering from diabetes are more prone to develop cardiovascular complications. Various epidemiological studies have shown patients with diabetes are more at risk to develop cardiomyopathy irrespective of age. Oxidative stress, *loss of cellular pro- and anti-oxidant balance,* is considered as a crucial factor associated with the propagation of diabetes and associated complications. Therefore, the present study was designed to investigate the role of Trolox (An analog of Vitamin E) pre-treatment on diabetic cardiomyopathy.

Materials and methods: Cultured H9c2 cells were exposed to glucose concentrations of 5.5 and 33mM for 24 and 72h with/without 100µM Trolox pre-treatment. At the end of each duration, cells were stained with H2DCFDA, Fluo-4, and mitotracker to determine the cellular ROS, intracellular Ca^{2+} load, and mitochondrial membrane permeability transition (MPT). Cytochrome-c levels were estimated by ELISA and levels of activated caspase-9, -3 along with Annexin-V/PI positive cells were determined by labelling them with specific conjugated fluorescent antibody and through flow cytometry.

Results: 33mM glucose exposure induced apoptosis in H9c2 cells by disrupting the redox state and MPT ($p<0.0001$). The obtained results demonstrated that 33mM glucose exposure enhance ROS generation ($p<0.0001$), and intracellular Ca^{2+} levels ($p<0.01$), and further activated the mitochondria-mediated caspase-dependent apoptotic pathway ($p<0.001$). The H9c2 cardiomyoblasts upon pre-treatment with Trolox exhibited a decrease in intracellular ROS and Ca^{2+} levels, restored MPT alongside inhibited the release of mitochondrial Cyt-c, thereby prevented caspase mediated apoptosis as confirmed by decrease in Annexin-V positive cells ($p<0.001$) when subjected to 33mM glucose. In addition, the present results also confirm the significant role of duration on glucose exposure induced apoptotic cell death in the H9c2- rat cardiomyoblast.

Conclusion: Trolox pre-treatment ameliorated high glucose induced cell death in H9c2 cells by restoring cellular redox balance.

PPBS-5

TITLE: Regulatory T- cells: the major immunological determinants in human visceral leishmaniasis

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Objective: Pathogenesis of Visceral Leishmaniasis (VL) is associated with suppression of T effector cell function. Immune cells such as Treg cell and its signature cytokines (IL-10 & TGF-β) dominantly suppress the T effector cell functions. In the present study, we evaluated the role of suppressive milieu (Treg cell, IL-10 & TGF-β) on pro-inflammatory cytokine production and their rescue.

Methods and methods: PBMCs were isolated from peripheral blood samples of parasitologically confirmed VL patients along with endemic controls. Isolated PBMCs were cultured and stained with monoclonal antibodies for flow cytometer based immunophenotyping. Serum from the study subjects were used for the evaluation of soluble level of cytokines.

Results: Our *in vitro* observation revealed significantly lower percentage frequency of pro-inflammatory cytokine producing cells; IFN-γ (CD4+ IFN- γ+) ($P<0.0001$), Th17 (CD4+ IL-17A+) ($P< 0.0002$) in VL patients as compared with healthy family controls. In contrast, the percentage frequency of Treg cells (CD4+ CD25+ Foxp3+) ($P<0.002$), anti-inflammatory cytokines producing cell: IL-4 ($P< 0.02$), IL-13 ($P< 0.062$), IL-10 ($P < 0.008$) and TGF-β ($P < 0.0032$) was significantly high in VL patients when compared with healthy family controls. Similar trends were also observed at the soluble level by ELISA. Furthermore, significant rescue of pro-inflammatory cytokine producing cells was observed upon neutralization of suppressive cytokines (IL-10 and TGF-β). IL-17RA expression on monocytes was also significantly low. Furthermore suppressive cytokines also down regulate IL-17RA expression on monocytes. Moreover, on all T cell subsets, IL-10R (CDw210) was also significantly high.

Conclusion: This study thus highlights that enriched Treg frequency with suppressive cytokine milieu inhibits the pro-inflammatory cytokines production and receptor expression.

PPBS-6

TITLE: Characterization of 1-2 µm diameter particles generated from agonist-stimulated platelets

Neha Rai, Debabrata Dash
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Objectives: Platelets are small, enucleated cytoplasmic fragments of megakaryocytes having diameter 2¬4 µm. They are known to generate extracellular vesicles (EVs) of varying sizes upon stimulation. Out of them, microparticles (100-1000nm) are most extensively studied. In our study we tried to characterize the platelet derived small-sized particles (1-2 µm diameter).

Materials and methods: Platelets were isolated from healthy volunteers by differential centrifugation. Platelets were co-stimulated with thrombin and collagen to generate the particles of varying sizes. 1-2 µm diameter particles were enriched by differential centrifugation. Fibrinogen binding as well as effect of RGDS (Arg-Gly-Asp-Ser) on fibrinogen binding was measured using flow cytometer. Statistical analysis was performed using GraphPad Prism 8 software.

Results: Platelet-derived particles of size 1-2 µm were found to bind more fibrinogen than the parent platelets. RGDS is a known competitive inhibitor of fibrinogen binding to αIIbβ3 on platelet surface. Small-sized particles pre-treated with RGDS significantly inhibited fibrinogen binding.

Conclusion: Platelet-derived particles of size 1-2 µm have greater affinity for fibrinogen than the parent platelets, suggesting that they have more integrins αIIbβ3 on their surface in active conformation. Similar to platelets (2-4 µm), fibrinogen binding to these particles is also inhibited with RGDS.

PPBS-7

TITLE: Flow cytometry based cell sorting – from immunology to developmental biology

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Objectives: The potential to separate cells into distinct populations has become the cornerstone in the field of research. Flow cytometry is an evolving dimension in the world of cell sorting. Our study highlights the applications of flow cytometry in the sorting of diverse cell populations and various cell types. It also accentuates the technical aspects that one should consider when sorting cells in terms of acquiring maximum purity and yield.

Materials and Methods: 3 different samples like peripheral blood mononuclear cells (PBMCs), TZM-bl cell line and mouse fetal gonadal cells (testis and ovaries) were sorted and different assays were performed using the sorted cells. The PBMCs were stained and CD4+ T cell subsets were sorted to study integrated HIV copies using Alu PCR. The expression of CD4 receptors in the cell line was reduced and so in-order to get an enriched population CD4 and CCR5 low and high double positives were sorted and further cultured in DMEM. To study the role of Lhx2 gene in sex determination, cells were isolated from mouse fetal gonads. Oct4 is a germ cell specific marker and therefore GFP was tagged under Oct4 promoter in Oct4-GFP transgenic mouse line, GFP+ (germ cells) and GFP- (somatic cells) were sorted, followed by RNA isolation and cDNA synthesis. In all the 3 experiments, cells were sorted using BD FACS Aria Fusion (SORP) using 70micron nozzle. Compensation was calculated and gating for each specific cell type was done on the basis of unstained sample and FMO. Accudrop and test sort was performed followed by 4-way and 2-way sorting wherein sorted cells were collected in tubes previously coated with media containing serum.

Results: The sorted CD4+ subsets had purity of more than 95% and DNA isolated from these subsets was positive for integrated HIV. Culturing of the sorted TZM-bl cells resulted in enrichment of CD4 expression from 40% to 60-70-%. With regards to gonadal cells, RNA isolated from sorted cells was found to be specific for the somatic and germ cells on performing qPCR using specific primers. The important technical aspects to be considered are; compensation which is calculated to overcome the spectral overlap and use of FMO control is critical when analysing rare cell population and cell activation markers on immune cells as in our case. Accudrop calculates the drop delay – the point at which the drop separates from the stream – it ensures that the instrument precisely places the charge on the drop containing the particle of interest. Test sort takes care that the drop falls into the collection tube which can be done by adjusting the voltages of stream during test sort. Coating the collection tubes with media containing serum enhances cell viability.

Conclusion: High purity sorting is vital, as it is the step that determines the success of the further assays of the study. Flow cytometry based sorting strategy is an important and widely applicable technology for research in diverse areas like developmental biology, cell culture and HIV pathogenesis.

PPBS- 8

ITLE: Flow cytometric assay for detection of prothrombotic COATed platelets in Healthy Volunteers

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Objectives:- Discovery of novel biomarkers is still of interest to predict emergency thrombotic states, the aim of this study was to investigate distinct platelet subpopulation, produced by a simultaneous activation of collagen and thrombin; COATed-platelets that represent a subpopulation of activated platelets with high Phosphatidylserine exposure and a substantial prothombinase activity in healthy individuals using flow cytometry.

Materials and methods: Healthy volunteers were enrolled and demographic data such as age, sex, smoking status, , past medical history and medications were noted. After obtaining informed consent, 5 ml of blood was drawn into sodium citrate vials and platelet rich plasma (PRP) was prepared. PRP was activated with thrombin-convulxin combination. The resulting platelet phenotype percentage of abciximab-positive events (platelets) with bound biotin-fibrinogen were quantitated by flow cytometry. Results are reported as percent of cells converted to COATed platelets.

Results: This study was carried on 20 adults(10 males and 10 females); average age 32.7 yrs ranging between 20 and 42. Mean platelet count was 244.6±92.3 x 10^9 per liter. The fraction of COATed-platelets yielded in healthy individuals was 24.4% with a range of 15.1% – 35%. COATed platelets levels were significantly elevated in males compared to females (24.9% vs. 22.1%; p 0.04)

Conclusion: Among normal blood donors, there is a large range of CAOTed-platelet potential as detected by flow cytometry. Future studies will hopefully be able to address physiological significance of these COATed platelets.

PPBS-9

TITLE: Viremic non-progressors preserve CD4+ central memory T cell homeostasis and maintain salutary CD8+ effector T cell population

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Objectives: The hallmark of HIV-1 infection is progressive CD4+ T cell depletion and extensive viral replication. Long term non-progressors (LTNPs) in HIV-1 infection are characterized by stable $CD4^+$T cell count for several years of infection (YOI) in absence of anti-retroviral therapy (ART). Recently, a rare group of LTNPs were observed who maintain high CD4 + T cell count despite high viral replication and also remain asymptomatic termed Viremic non-progressors (VNPs). The current study aimed at immune characterization of VNPs to better understand mechanisms in coping with viral pathogenesis and identify novel strategies for immune restoration under ART-failure.

Material and Methods: In this cross sectional study, 21 HIV seronegative individuals (SN) and 48 HIV-1 infected participants including VNPs (N=18), Putative Progressors (PuPs, N=14), standard progressors (SPs, N=8) and Viremic controller (VCs, N=8) were recruited for this study from ART Centre at Sir J.J. group of Hospitals, Mumbai. All the study groups except SPs was recruited with $CD4^+$T cell count ≥500 cells/µl. VNPs with plasma viral load (VL) >10000 copies/ml, ≥7 YOI and PuPs with VL >2000 copies/ml and < 3 YOI along with SPs (>2000 copies/ml; < 3 YOI) and VCs (< 2000 copies/ml and > 7 YOI) were studied for different key immune parameters using flow Cytometry.

Results: In the current study, VNPs were recruited using stringent criteria and were similar toPuPs with respect to CD4 count, CD8 count, CD4/CD8 ratio and viral load and allowed robust comparison for homeostatic immune signatures. We found elevated immune activation of both CD4 and CD8 T cell compartment across all the study groups except for VCs. Intriguingly, despite high systemic immune activation we observed elevated Treg frequency and positive association of immune activation and Treg frequency exclusively in VNPs. We also observed stable CD4+ central memory T cell compartment (CD4 T_{CM}) compared to PuPs and SPs despite years of HIV infection. VNPs also had preserved thymic function. Furthermore, a differential regulation of CD8+ effector population was observed where VNPs have preferential diminution of terminally differentiated CD8+ T cells.

Conclusion: In our study, VNPs demonstrated an apparent compensatory role of Treg for attenuation of effects of immune activation. VNPs phenotype was also associated with preserved CD4 T_{CM} compartment possibly contributed by preserved thymic function to resist CD4+ T cell depletion. In addition, preferential diminution of terminally differentiated CD8+ T cells might ameliorate consequences of high viral replication.

PPBS-10

TITLE: Dendritic cell vaccines for HPV positive cervical cancer and the effect of cisplatin on autologous tumor lysate pulsed DC vaccines

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Objective: Human Papilloma Virus (HPV) is linked to 99.7% of cervical cancer incidence and its presence causes immune perturbations which lead to disease progression. However in advanced cervical cancers (stage III and above), conventional chemotherapy can cure only 50% of the cases. Hence an immunotherapeutic adjuvant could boost anti-tumour immunity. In this study, we evaluated the efficacy of tumour lysate-pulsed DCs (TLDCs) in terms of their phenotype and functionality in the presence and absence of cisplatin.

Materials and methods: Peripheral blood and punch biopsy samples from 20 cervical cancer patients were obtained for the study.

Results: We found that TLDCs could significantly ($p=0.03$) stimulate autologous lymphocyte populations and maintain their efficacy in the presence of cisplatin. TLDCs were capable of stimulating autologous PBMCs to secrete Th1 cytokines (IL12p40, IL12p70 and IFNγ) even in the presence of cisplatin at 200μM concentration.

Conclusion: Our results indicate that at although at 200μM concentrations, cisplatin did reduce the viability of DC, this reduction was not significant and it also did not affect the functionality of TLDCs. Hence combining dendritic cell vaccination along with cisplatin-based chemotherapy may be effective to induce a productive response in cervical cancer.

PPBS 11

TITLE: Dendritic cells (DCs) Quantification and functional analysis in peripheral blood during hepatitis C infection with different disease category

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Introduction: An estimated 3% of the world's population is infected with hepatitis C virus (HCV). With low spontaneous clearance rates by the host immune system, approximately 85% of infected patients have increase risk for developing liver cirrhosis and hepatocellular carcinoma. The mechanisms of HCV persistence remain elusive. Myeloid Dendritic Cells (MDCs) play an important role in the initiation of adaptive antiviral immune response by acquiring and processing antigens for specific T cells. In addition, Plasmacytoid Dendritic Cells (PDCs) participate in antiviral responses by producing large amounts of IFN-a/b. Present study was designed to rule out the role of MDCs and PDCs in HCV infected patients.

Material and methods: Total of 125 patients (Anti-HCV positive with no viral load (n=25), Acute HCV (n=25), chronic HCV (n=25), Cirrhosis with HCV (n=25) and HCV related HCC (n=25)) and 25 age and gender matched control were enrolled in the present study. HCV load was quantified from serum samples by use of real-time polymerase chain reaction (PCR). For DCs analysis, PBMCs were isolated by use of density-gradient separation using FicollHypaque (Pharmacia) and analyzed by using flowcytometry with DCs specific antibodies.

Results & Discussion: Hepatitis C infected patients showed a lower absolute cell count percentage of peripheral blood mDCs when compared to healthy controls, while only antibody positive HCV patients did not display any statistical significant differences with healthy individuals, thereby suggesting the lack of enrollment in peripheral blood of this dendritic cell subset during the HCV infection.

Conclusion: Our results showed that during hepatitis C, the virus might prevent the activation of circulating myeloid dendritic cells (ILT3/CD11c+) conditioning the natural history of the hepatitis. Thus, an early assessment of mDCs using enumeration strategy could give useful suggestions about the possible progression of infection, thereby proposing the early enrolment of the patients in treatment.

Poster Presentation- Clinical Science (PCS)

PPCS -15

Title: Immunophenotypic profile of plasma cells in newly diagnosed multiple myeloma

Priyanka Gupta, Ruchi Gupta, Khaliqur Rahman, Seema Biswas, Dinesh Chandra, Manish K Singh, Sanjeev, Anshul Gupta, Soniya Nityanand.

Institution: Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow

Objective: Flow cytometric immunophenotyping has been used to identify neoplastic plasma cell populations in patients with multiple myeloma (MM). Reports have described the use of several antigens, including CD38, CD56, CD117, CD52, CD19 and CD45 to identify abnormal plasma cells. The aim of this study was to evaluate the immunophenotypic profile of plasma cells in newly diagnosed patients of multiple myeloma.

Material and Methods: Bone marrow samples of 62 newly diagnosed patients of multiple myeloma were subjected to flow cytometry. The samples were processed by a lyse-stain–wash protocol. Pre-titrated antibody cocktails comprising of CD138(FITC), CD38, CD19 (PE-Cy-7), CD45(V500), CD28(Per-CPCy5.5), CD81(APC-H7), CD56(APC), CD117(V450) and CD200(PE) were applied. A minimum of 3×10^3 plasma cells were acquired on BD FACS Canto II or FACSLyric flow cytometer and analyzed with FACS DIVA and FACSuite analysis software (BD, Biosciences, San Jose CA, USA) respectively.

Results: The median age of patients was 60 years with a M:F of 3.2:1. Median bone marrow plasma cell percentage as assessed on MGG stained bone marrow smear was 52% (range 10-93%) and 17% (range 3.6-93.2%) in samples subjected for flow cytometry. Serologically, 22 had IgG kappa, IgG lambda in 11, IgA kappa in 2, IgA lambda in 5 patients was seen. Light chain myeloma was present in 14 cases (kappa light chain, 6 and lambda light chain 8). In adjunct to CD38/CD138, CD229 was found to be a suitable gating marker in all the cases. Aberrant expression of CD200, CD28, CD56, CD81 and CD117 were observed in 74%, 29%, 92%, 16% and 37% cases respectively. Dim expression of CD45 and CD19 in these abnormal plasma cells was noted in 6% and 29% cases respectively. Renal involvement was present in 27/ 62 patients, of which loss of CD117 expression was noted in 14/27 cases.

Conclusion: A combination of CD56+/CD200+/CD45- in adjunct to gating markers CD138 and CD38 can identify abnormal plasma cells in majority (>75%) of the cases with plasma cell dyscrasia.

PPCS-16

TITLE: Ploidy analysis of plasma cells in newly diagnosed plasma cell dyscrasias and their correlation with other prognostic factors

Seema Biswas, Ruchi Gupta, Khaliqur Rahman, Priyanka Gupta, Dinesh Chandra, Manish K Singh, Sanjeev, Anshul Gupta, Soniya Nityanand.
Institution: Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow

Objective: Risk stratification by cytogenetic analysis plays an important role in predicting treatment response in myeloma. While cytogenetic testing is tedious, ploidy analysis coupled with multiparametric flow cytometry also provides valuable information about the genetic make-up of the plasma cells and their behaviour. The aim of the study was to perform ploidy analysis in newly diagnosed myeloma patients and correlate their status with other laboratory and clinical parameters.

Material and Methods: Bone marrow samples of newly diagnosed patients of multiple myeloma were subjected to flow cytometry within 24 hours of sample collection after morphological evaluation. The samples were processed by a lyse-stain–wash protocol. Pre-titrated antibody cocktails comprising of CD138, CD38, CD19, CD45, CD28, CD81, CD56, CD200, and DNA ploidy dye, FxCycle Violet (Fx) were applied. A minimum of $3x\ 10^3$ plasma cells using CD138/CD38 were acquired. Samples were run on BD FACS Canto II flow cytometer as well as FACSLyric and analyzed with FACS DIVA and FACSuite analysis software (BD, Biosciences, San Jose CA, USA) respectively.

Results: The median age of patients was 60 years with a M:F of 3.2:1. Median bone marrow plasma cell percentage as assessed on MGG stained bone marrow smear was 52% (range 10-93%) and 17% (range 3.6-93.2%) in samples subjected for flow cytometry. Ploidy analysis revealed hyperploidy in 61.2% cases (38/62). Out of total 38 cases of hyperploidy; renal involvement was found in 50% of cases (19/38). Loss of CD117 and hyperploidy was noted in 57.8% (11/19) of cases of renal involvement showing negative CD117 expression.

Conclusion: Ploidy analysis coupled with immunophenotype is a useful tool to predict disease severity and renal impairment in myeloma, which should be incorporated in routine clinical practice.

PPCS-17

Title: Functional perturbation of monocyte subsets in chronically HBV infected patients with high levels of hepatitis B surface antigen and its persistence after antiviral therapy

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Objective: Chronic HBV infection (CHI) is a major cause of end-stage liver disease for which pharmacological treatments currently available are inadequate. Chronically HBV infected patients fail to mount an efficient immune response to the virus, impeding viral clearance and recovery from hepatitis. Monocytes are critical components of innate immunity that not only help in the control of microbial infection but also contribute to pathogenesis of inflammatory diseases. However, a comprehensive understanding on monocyte involvement in CHI is still lacking. To investigate the frequency/phenotype/function of monocyte-subsets in all phases of CHIImmune-tolerant (IT), HBeAg-positive/HBeAg-negative (EP/EN) chronic hepatitis B (CHB) and Inactivecarriers (IC) and to examine the effect of antiviral therapy on these cells.

Materials and methods: Monocyte-subsets were analyzed by flow-cytometry. FITC-Zymosan particles and DCFH-DA were used to study phagocytosis and cellular ROS respectively. Phenotype/function of monocytes was longitudinally monitored in Tenofovir-treated CHB patients.

Results: HLA-DR+CD14++CD16-classical-monocytes were significantly reduced while HLA-DR+CD14++CD16+intermediate- and HLA-DR+CD14+CD16++ non-classical-monocytes were enhanced in EP/EN-CHB than I Cand healthy controls (HC). In comparison to IC/HC, all monocyte-subsets in IT and EP-/EN-CHB exhibited diminished TLR-2/TLR-4 and IL-6/IL-12 expression but higher levels of TGF-β and IL-10, which correlatedwith high hepatitis B surface antigen (HBsAg) concentration in IT/CHB patients. A significant decrease in phagocytic potential of monocyte-subsets, as evident from CD64 expression and engulfment of FITC Zymosan particles and reduced generation of ROS were observed in IT/CHB than IC/HC. Further, monocyte-subsets in IT/CHB, expressing reduced intracellular iNOS but elevated Arginase-1, showed high propensity to skew toward immune-suppressive M2-phenotype. One year Tenofovir therapy failed to normalise monocyte functions or reduce serum HBsAg levels despite reduction in HBV-DNA.

Conclusion: Collectively, monocyte-subsets are functionally perturbed in IT/CHB patients having high HBsAg concentration. Hence, therapeutic targeting of monocytes or reducing circulating HBsAg might be effective in restricting HBV persistence and disease progression.

PPCS-18

TITLE: Clinical significance of end induction minimal residual disease monitoring in B cell acute lymphoblastic leukemia: a single centre experience

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Institution: Christian Medical College, Vellore.

Objectives: Assessment of minimal residual disease (MRD) has emerged as a powerful prognostic tool in both paediatric and adult acute lymphoblastic leukemia (ALL). In this retrospective study, we aimed to evaluate the prognostic relevance of MRD in B ALL patients.

Materials and methods: The study included 403 patients who underwent treatment for B ALL between August 2012 and March 2019 in the Department of Haematology, CMC Vellore and had their end induction MRD assessed by flow cytometry. MRD assessment was done by 4 tube-4 colour panel (till July 2017) and 2 tube-8 colour panel (from August 2017). MRD levels $\geq$ 0.01% were considered positive. Baseline demographic characteristics were collected from the patient's clinical records. Statistical analysis was done using SPSS version 16.0 software. (SPSS, Chicago, IL)

Results: Compared to the 4 tube-4 colour panel, the 2 tube 8 colour panel had wider applicability (98.5% vs 93.2%) and showed a positive MRD in a higher percentage of patients (43.08% vs 30.48%; p=0.046). End Induction MRD had a significant impact on Relapse Free Survival (RFS 34.1%$\pm$8.5% vs 83.9%$\pm$3.4%; p<0.001) and Event Free Survival (EFS 29.8%$\pm$7.6% vs 81.6%$\pm$3.5%; p<0.001) and the survival worsened for every log increase in the MRD values. Among the other variables, older age, poor prednisolone response, presence of *BCR-ABL1* translocation and poor risk cytogenetics were significantly associated with poor survival while *TEL-AML1* translocation showed a trend towards better EFS (p=0.097). On multivariate analysis positive MRD showed the strongest association with both RFS and EFS.

Conclusion: End induction MRD was the strongest prognostic indicator in B-ALL patients among the different parameters assessed in our study. End induction MRD is the ideal tool to predict treatment responses and take necessary clinical interventions including the need for a bone marrow transplant.

PPCS-19

TITLE: Clinical utility of multicolor flowcytometry in the diagnosis of malignant (metastatic) serous effusions

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Institution: Tata Memorial Hospital, Mumbai

Objective: Multicolor flowcytometry (MFC) is extensively used in the management of hematological neoplasms. However, its clinical utility in non-hematological malignancies is sparse. Epithelial antigen EpCAM and CD44 are commonly expressed in most of the epithelial cells (genitourinary, gastrointestinal, hepatobilliary, and breast tissue). In normal serous cavity fluids epithelial cells are not seen. We standardized a 10-color single-tube MFC-assay using simultaneous analysis for surface-staining of EpCAM (CD326), CD44 (cell surface adhesion receptor) and DNA-ploidy analysis for the rapid and Objective diagnosis of metastatic serous effusions (MSE).

Methods: Total 48 samples of serous body cavity fluids either from patients with clinical suspicion of malignancy or in a known case of carcinoma were processed with a 10-color panel including antibodies against CD45, CD11b, CD13, CD44, CD325, CD326 and FxCycle-violet dye (FCV). MFC was performed on Cytoflex (Beckman-Coulter) flow-cytometer and data-analysis was performed using Kaluza-v2.1-software. MFC-results were compared with cytomorphology and cell block morphology or immunocytochemistry wherever available.

Results: Total 48 suspected body fluid samples were processed from patients suspicious for MSE (age range, 2-72 years and M:F ratio 1:1.4). A novel gating strategy was developed. According to which contaminating blood cells (lymphocytes, monocytes, granulocytes and macrophages) were excluded using CD45, CD11b and CD13. Tumor cells were identified using the positive expression of CD44, CD325 and CD326 and then DNA-ploidy analysis with FCV. Of 48 fluids, 38 were involved in cytomorphology studies. Out of 38, 30 (80%) were EpCAM positive and 28/38 (74%) were also positive for CD44 but CD325 didn't show positive expression. In MFC could not detect the tumor in eight samples due to reasons like the formation of clots and scarce samples. DNA Ploidy analysis was available in 28/38 cases and aneuploidy was detected in 26/28 cases. In two-cases, ploidy analysis did not work satisfactorily due to a technical issue.

Conclusion: We standardized a novel 10-color MFC-panel that allowed the rapid diagnosis of metastatic serous effusions using simultaneous analysis of six-surface markers and DNA-ploidy study. CD326, CD44 but not CD325 are very useful markers in the detection of metastatic epithelial tumor cells.

PPCS-20

TITLE: Standardization of high sensitivity minimal residual disease monitoring in multiple myeloma: an experience in tertiary cancer centre

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Institution: Tata Memorial hospital, Mumbai

Objective: Minimal residual disease(MRD) status is the most relevant prognostic marker in multiple myeloma (MM). Recently, IMWG has incorporated MRD-negative status as a criterion to define stringent clinical response(sCR) using next-generation flow cytometry(NGF) i.e. high-sensitivity flow cytometry (HSFC). Herein, we present our experience in the standardization of HSFC and MRD monitoring in MM.

Materials and Methods: We standardized HSFC MRD-assay in MM using Euroflow bulk-lysis and stain method. A ten-color two-tube antibody panel was used which included antibodies against CD19, CD20, CD27, CD28, CD38, CD45, CD56, CD81, CD117, CD138, CD229, CD319 and Cytoplasmic-kappa and lambda. Sample were acquired using Navios flow-cytometer and data was analyzed using Kaluza-software. Limit of detection (LOD) and lower limit of quantitation (LLOQ) were determined using spiking and dilution experiments.

Results: LOD and LLOQ ofthe HSFC-MRD was found to be 10 events (sensitivity of 0.0003%) and 25 events (sensitivity of 0.0008% with CV of 23.8%). We studied HSFC-MRD in 128 bone marrow samples from 99 MM patients (age- median-54 years, range-29 to 74 years and M:F ratio-4.2). Number of cells studied for MRD ranged from 700,000-9,900,000 with median of 3,400,000. MRD was detectable in 62.7% (79/128) samples and MRD levels ranged from 0.0002% to 23.4% with median 0.2%. Correlation with serum M protein showed a correlation coefficient of 0.59 with 12% cases showing MRD positivity without detectable M protein. CD19, CD45, CD27, and CD56 demonstrated highest frequency of abnormal expression in MRD detection (i.e. 100.0%, 90.9%, 87.0%, and 87.0%), followed by CD117, CD200, CD81, CD28, CD38, and CD20 in decreasing order (i.e. 62.3%, 54.5%, 50.6%, 35.1%, 16.9% and 10.4%). Median (range) of LAIP detected in MRD was 6(2-8) using these markers.

Conclusion: We standardized a highly sensitive 10-color flow cytometric MRD assay for MM. These essential markers must be included in the MM-MRD panel.

PPCS-21

TITLE: **Role of *neutrophil CD64, monocyte HLA DR* and *sepsis index* as newer biomarkers for diagnosis of sepsis in adult patients**

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Objective: Neutrophil CD64 (nCD64), Monocyte HLA DR (mHLADR) and Sepsis index (SI=nCD64/mHLADR X100) were evaluated as prospective biomarkers for sepsis diagnosis and prognosis in adult patients.

Material and Methods: Adult patients admitted in (ICU n=30) were included in the study. Acute change in SOFA score of 2/more with clinical suspicion and/or culture positivity formed the basis of sepsis diagnosis. Whole blood sample was collected on the day of admission for nCD64, mHLADR, cultures and other relevant parameters for patient management in ICU. Blood samples from fifteen blood donors were collected as healthy controls. nCD 64 and mHLADR were quantitated as ABC (antibody bound per cell)by BD Quantibrite™ reagents as per the manufacturer's instructions on BD FACS Canto using BD FACSDiva software.

Results: Cases were categorized into Sepsis n=22 (culture positive n=15, culture negative n=7), Non-sepsis n=8 (disease control) and healthy control n=15. Median values(Q1-Q3) for nCD64 for sepsis, disease control and healthy controls were 2258(1237-3584), 999.5(527-1114) and 348(243-467); for mHLADR were 2682(1627-5560) ,9292(5966-12440) and 14950(11604-19466); and for SI were 74.75(42.80-190.7), 8.050(5.47-17.78) and 2.10(1.7-2.7) with significant difference between the 3 groups (sepsis vs. disease controls: p= 0.002 (nCD64), 0.003 (mHLADR), 0.0001(SI). Diagnostics by ROC curve analysis for Sepsis vs disease control were as follows: nCD64 at cutoff >1205, p 0.002, Sensitivity 77.27%, specificity 87.5%; mHLADR at cut off < 3717, p 0.003, Sensitivity 68.18%, specificity 100.0%; SI at cut off > 40.35, p 0.0001, Sensitivity 81.82%, specificity 100.0%. Median values for all three parameters were significantly different between survivors (n=14) and non survivors (n=16) in the sepsis and disease control group.SI gave 100% sensitivity in predicting mortality followed by nCD64 and mHLADR.

Conclusion: nCD64, mHLADR and SI appear to be promising biomarkers for differentiating patients of sepsis from those who do not have infection as well as for predicting mortality.

PPCS- 22

TITLE: Flow cytometry based study of *CRLF2* expression in B-ALL : an experience from a tertiary care center.

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Objective: Cytokine receptor–like factor 2 (*CRLF2*) gene is located in the pseudo autosomal region 1 (PAR1) at both sex chromosomes, X (Xp22.3) and Y (Yp11.3). *CRLF2* genomic rearrangements are found in approximately 50% of Ph-like B-cell acute lymphoblastic leukaemia (B-ALL). *CRLF2* rearrangements are often associated with its overexpression and can be detected by flowcytometry using the antibody against TSLPR - thymic stromal lymphopoietin receptor (TSLPR) and by real time PCR. We share our experience of evaluation of *CRLF2* expression using the flowcytometry based assay.

Material and methods: A prospective *CRLF2* expression analysis using BV510 mouse antihuman TSLP receptor (BD horizon™, clone: 1F11/TSLPR) was done in 160 consecutive cases of B-ALL (146 diagnostic, 14 relapsed) by flow cytometry (BD FACSCanto™II) from February 2018 to May 2019. Karyotyping and Interphase FISH analysis using *CRLF2* gene break-apart probe was carried out in cases which had *CRLF2* overexpression (cut-off >10%).

Results: Of the total 160 cases evaluated (age range 1 year to 75 years, Male: Female ratio 1.6); 12.5% cases (n=20) overexpressed *CRLF2*. Majority of the cases (15/20) had CRLF2 expression >50%. These 20 cases were diploid (n=13) and hyperdiploid (n=6) by flow ploidy. Seventeen cases were diagnostic (12.1 %, 17/140) whereas three five presented as relapsed B-ALL (25%, 5/20). Overall In adults (>15 yrs) 17% (8/47) cases had *CRLF2* overexpression (Diagnostic 15%; 6/40) while in paediatrics*CRLF2* overexpression was seen in 12% (12/100) B ALL cases (Diagnostic 6.4%; 6/93). Cytogenetic analysis performed in 19/20 cases revealed *CRLF2* rearrangement in four cases. The rest thirteen *CRLF2* FISH negative cases included hyperdiploid (n=7), t(8;22)(n=1), t(1;19)(n=1), t(9;22)(n=1) and *KMT2A* gene rearrangement(n=1). Normal karyotype was found in remaining two cases. Post induction (day +28-35) MRD status was available for thirteen newly diagnosed cases and 53.8% (7/13) had a positive MRD. Two adult cases died within 1 month of the diagnosis and one relapsed in paediatrics.

Conclusion: Flowcytometry based analysis of *CRLF2* over expression is rapid, inexpensive and simple technique in triaging the cases of B-ALLs to further investigate for *CRLF2* rearrangements by other techniques. *CRLF2* by flowcytometry can be included in diagnostic algorithms of B-ALL as a screening tool for further investigation.

PPCS-23

TITLE: Study of the expression pattern of B-cell maturation antigen (BCMA, CD269) in multiple myeloma using multicolor flow cytometry

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Objective: Multiple myeloma (MM) is an incurable malignancy of plasma cells (PCs). Recently, the cell-surface protein B-cell maturation antigen (BCMA, CD269) has emerged as a promising target for CAR-T cell and monoclonal-antibody therapies in MM. However, the knowledge of the expression-pattern of BCMA on PCs is still scarce. We present an in-depth study of the expression pattern of BCMA in abnormal-PCs from MM.

Methods: We analyzed expression-pattern of CD269 (PE Dazzle 594 clone-19F2) in clonal-PCs from newly diagnosed MM. MFC characterization was performed on Cytoflex (Beckman-Coulter) flow-cytometer and dataanalysis was performed using Kaluza-v2.1-software. Expression-levels of CD269 in PCs was determined as a mean fluorescent intensity (MFI) using geometric mean. The pattern of expression (homogenous/heterogeneous) was determined as coefficient-of-variation of immunofluorescence (CV-IF).

Results: We studied BM samples from 80MM patients with a median age of 58.7 years (range,37-78years)and M:F- 1:9. PCs were gated using a novel reference gating strategy using the combination of CD38 vs CD319 vs CD45. Median of flow cytometric percentages of PCs was 3.13% (range, 0.6-37.5%). Median (range) percentage of CD269-positive abnormal-PCs in total gated PCs was 81% (27-100%). CD269 negative in >50% (27-49%) PCs In 7/80 (8.8%) samples, in >25%<50% (27-49%) PCs In 22/80 (27.5%) samples and <75% in remaining (67.3%) samples. Median (range) of MFI and CV-IF of CD269 in MM-PCs was 3.4 (1.1-11.2) and 62.55 (34.5-124.3). In 62/80 (78%) samples, CV-IF of CD269 in MM-PCs was <80 (median, 56.7 and range, 34.7-77.8) indicating its homogenous expression in the majority of cases.

Conclusion: In this study, we demonstrated the expression-pattern of BCMA (CD269) in abnormal-PCs from MM. CD269 showed the bright and homogenous expression in 80% of MM samples. This study highlights the value of CD269-protein as a promising target for newly developed targeted therapies in most of the MM patients.

PPCS-24

TITLE: Standardization of flow cytometric staining of Ki-67 expression in B-cell non-Hodgkin's lymphoma

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Objective: Ki-67 is a nuclear protein associated with cellular proliferation in normal or leukemic conditions that can help identify more aggressive diseases and essentially used as a prognostic and/or predictive biomarker in non-Hodgkins lymphoma (NHL). It is usually evaluated by immunohistochemistry (IHC), however IHC interpretation can be influenced by the presence of non-malignant reactive, variable sample-processing methodologies, and interoperator variations. Flow cytometry (FC) is extensively used for Objective diagnosis and classification of hematological neoplasms. However, flow cytometric staining of Ki-67 is not standardized for clinical utility. We have standardized the FC-protocol for the staining of Ki-67 and evaluated their expression pattern in B-NHL.

Methods: We standardized and studied FC-staining of Ki67 (PE, clone-REA183, MiltenyiBiotec) in peripheral blood, bone marrow, lymph node and FNAC samples from B-cell NHL. Cells were stained with typical surface markers used in B-NHL including CD5, CD10, CD19, CD20, CD38, and CD45 followed by cytoplasmic staining of Ki67. For cytoplasmic processing, we evaluated four different kits i.e. Fix-and-Perm (Invitrogen), FACS-lyse (BD), Foxp3 Transcription Factor Staining Buffer Set (eBioscience), and True-Nuclear Transcription Factor Buffer Set (Biolegend). FC was performed on Navios (Beckman-Coulter) flow-cytometer and data-analysis was performed using Kaluza-v2.1-software.

Results: Of the four fixation and permeabilization kits, FACS-lyse was found best for Ki-67 staining with 30 minutes of incubation. We studied 55 samples (PB-7, BM-45 and FNAC-3) submitted for the diagnosis of NHL. Ki-67 was studied in all 51 samples. The results of expression of Ki-67 is given in the Table-1.

Conclusion: We have standardized the flow cytometric protocol for analysis of Ki-67 expression in B-cell NHL. This protocol allows for Objective and reproducible evaluation of the proliferative index of B-cell NHL by flow cytometry for the assessment of disease severity and limits the need for immunohistochemistry on biopsy samples.

PPCS-25

TITLE: Flow cytometric immunophenotypes of DNMT3A, IDH1 AND IDH2 mutated de novo AML cases – a study of cases diagnosed in one and half year period in a tertiary referral center

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Insitution: 1-Ex DM hematopathology fellow, 2-Professor & Head, 3-Associate Professor, Department of hematology, 4-Professor & Head, Department of MedicinePost Graduate Institute of Medical Education and Research, Chandigarh

Objective: To determine the prevalence of DNMT3A and IDH1 and IDH2 mutations among newly diagnosed AML cases and their correlation with immunophenotypic features

Materials & methods: The study was conducted in the Department of Hematology, PGIMER, Chandigarh from January 2016 to June 2017. 103 de-novo AML patients were included in the study. Complete blood counts, bone marrow examination and cytochemical staining were done. Flow cytometric immunophenotyping was performed on BD FACS CANTO II. A primary panel consisting of CD 45, CD13, CD33, CD34, CD117, CD36, CD19, CD20, CD19, CD79a, cCD22, CD3, CD7, cCD3, CD8,TdT, MPO and HLA-DR were used. Additional markers like CD11c, CD15, CD56, CD2, CD9, CD56, CD14, CD64, CD41, CD61, CD71, CD235a etc. were used whenever required. Extraction of genomic DNA for molecular genetic analysis was performed using 'QIAamp® DNA blood Midi kit, Qiagen, Germany. Restriction fragment length polymorphism method, amplification-refractory mutation system and high resolution melting curve analysis method were used to identify DNMT3A R882H G>A mutation, IDH2 R140Q and IDH1 R132 mutations respectively. Appropriate statistical tests were performed and analysis was conducted using IBM SPSS STATISTICS (version 22.0).

Results- DNMT3A R882H, IDH1 R132 and IDH2 R140Q mutation was detected in 9 (8.7%) , 9 (8.7%) and 13 (12.6%) cases respectivelyImmunophenotypes of blasts of DNMT3A R882H mutated cases were CD13+, CD33+, CD117+, CD38+, CD34+/- and HLA DR+/- in >80% cases. Compared to wild type, the mutated cases were more frequently MPO negative (80.9% versus 44.4%). >80% of cases of IDH1 R132 mutated AML cases expressed CD13, CD117, CD38, MPO and CD33. However, CD34 was positive in only 33.3%. 15% of IDH2 R140Q mutated cases had significant dim cCD22 (p value <0.05).

CONCLUSIONS:IDH2 R140Q mutated blasts expressed aberrant cCD22 significantly compared to unmutated cases (p value=0.04) whereas there were no significant differences in antigen expression between the wild type AML and mutated DNMT3A R882H and IDH1 R132 AML cases

PPCS-26

Title: Study of the expression pattern of CD147 (EMMPRIN) in multiple myeloma using multicolor flow cytometry

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Objective: CD147 (extracellular matrix metalloproteinase inducer, EMMPRIN), a transmembrane glycoprotein, is expressed by many different cell types. Its over-expression is associated with increased angiogenesis, tumor growth, lower progression-free survival and poor clinical outcome in non-hematological cancers. The abnormally elevated levels of soluble-CD147 have also been suggested to be associated with poor outcome in MM. We investigated the expression-pattern of CD147 in MM and normal-PCs using multicolor flow cytometry (MFC).

Materials and methods: We analyzed expression-pattern of CD147 (BV605, clone-HIM6) in clonal-PCs from newly-diagnosed MM and normal-PCs from control samples (uninvolved staging BM). Expression-levels of CD147 in PCs were determined as a normalized mean fluorescent intensity (MFI) and it was determined as the ratio of MFI in PCs to CD147-negative lymphocytes (denoted as nMFI). The expression-pattern (homogenous/heterogeneous) was determined as coefficient-of-variation of immunofluorescence (CV-IF).

Results: Expression-pattern of CD147 was studied in 85 MM and 15 control samples. Median (standard deviation, SD) of total PCs/viable cells (TPCs) in MM and control samples were 6.6% (11.5%) and 0.58% (0.57%). Median (range) of nMFI of CD147 in clonal-PCs and normal-PCs was 8.1 (2.8-18.5) and 5.7 (3.4-7.5). Clonal-PCs had significant overexpression in comparison with normal-PCs ($p<0.05$). CD147 showed overexpression (nMFI above upper-level of normal-PCs) in 52/85 (62.16%) cases. Median and SD CV-IF of CD147 in PCs was 59.49 and 12.02, indicating its homogenous expression. Median (SD) of CD147-positive plasma cells (out of total-PCs gated with CD38vCD138vCD45) was 98.9% (6.17%). Median (range) of CD147-nMFI in rest of non-PCs hematopoietic cells (HCs) was 2.8 (1.4-5) and the expression-level of CD147 in PCs was revealed to be brighter than HCs ($p<0.001$).

Conclusion: CD147 showed the bright and homogenous expression in PCs as compared to rest of hematopoietic cells and the expression levels of CD147 was higher in clonal-PCS than normal-PCs and was found overexpressed in 62% of MM.

PPCS-27

TITLE: Flowcytometric MRD assessment in B cell acute lymphoblastic leukemia: a tertiary care experience of 500 samples

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Objective: Flowcytometric measurable residual disease (MRD) analysis is an important prognostic indicator of outcome in B-cell acute lymphoblastic leukaemia (B-ALL). We share our experience of standardized 8-colour flowcytometric MRD performed on 500 bone marrow samples in B-ALL.

Methods: This is a retrospective analysis and includes bone marrow samples processed from March 2016 to June 2019. We performed single tube/two tube, eight colour MRD assay for the post treatment disease assessment. This included backbone markers(CD38-FITC, CD34-PerCPCy5.5, CD10-PE-Cy7, CD19-APC, CD45-APC-H7, CD20-V500c) and discriminatory markers like CD58-PE, CD123-BV421 in tube one and CD81-PE, CD73-BV421 in tube two. We used the standard bulk erythrocyte lysis followed by antibody staining and acquisition of one million-events/tube (minimum 0.5 million). The laboratory participates in UKNEQAS for BALL MRD since 2016.

Results: A total of 500 samples from 351 patients were processed during this period**.** In majority of samples (95.4%) one million events were acquired in each tube. Of the total samples (154- single tube, 346-two tubes) evaluated 279(55.6%) were post induction (PI; day 35-42), 163(32.6%) were post consolidation (PC; Post counts recovery), and 59(11.8%) were subsequent follow-up. The MRD positivity (>0.01%) was 28.5%(59/207) in Paediatric PI samples and 46.5%(33/71) in adults. Overall any positivity was seen in 36.9%(103/279) of the PI samples (Paediatric=32.9%:68/207; Adults=48.6 %;35/72) and 34/163(20.8%) in the PC samples. MRD in high risk subgroup was 45.3%(39/86), 38.2%(26/68) in standard risk subgroup and 31.9%(30/94) in the intermediate subgroup. The most consistent Leukemia-Associated Immunophenotype (LAIP) in the PI samples was over-expression of CD73, followed by under-expression of CD38 and CD81.

Conclusion: Flowcytometric MRD measurement is an important tool in the treatment strategy of B-ALL patients. An optimized, cost-effective and validated panel is invaluable for an accurate detection of MRD and necessary treatment intervention(s). Constant modifications and standardization is warranted in improving this relatively simple and rapidly emerging technique in patient management.

PPCS-28

TITLE: Prognostic significance of Minimal Residual Disease (MRD) detection in B- ALL – Experience of a Standalone Flow Cytometry Laboratory

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Objective: In addition to standard risk criteria at diagnosis, Minimal Residual Disease (MRD) following initiation of therapy is a well-recognized risk factor to predict relapse in B-Acute Lymphoblastic Leukemia (B-ALL). Post induction MRD in B-ALL is now used as a prognostic indicator and also as a treatment modifier based on the treatment protocol and level of MRD detected. The lack of availability and standardization of the MRD assay across different labs represents a major challenge to utilization of MRD monitoring for the treating physician.

Methodology and Results –A total of 650 patient samples were evaluated for MRD over the last 24 months. Amongst all MRD cases, 526 cases had come for evaluation of B-ALL MRD and 124 cases had come for T-ALL MRD analysis. For B-ALL MRD assays, a single tube 8-colour panel (CD19/CD10/CD20/CD34/CD38/CD58/CD45&CD123) was used for these cases. Routine sensitivity for MRD assay was standardized based on a serial dilution assay at 0.01%. To increase limit of detection of MRD events – in all cases received event acquisition rates were increased upto 2 million events. MRD positivity was identified based on a cluster of minimum 20 (LLOD) cells forming a tight cluster and showing deviation from the normal maturation patterns and levels of antigen expression compared with normal or regenerating marrows. Being a referral flow cytometry laboratory we received a mix of pediatric (405/526) and adult samples (121/526) with patients being treated under different protocols like BFM, COG, UK-ALL and ICICLE protocols. Majority of the samples received were at the post induction stage with the rest being at varied treatment time points. A total of 162 MRD positive cases (~30.79%) with MRD levels ranging from 0.0006% to 94% were identified. In order to obtain homogenous data, a single centre study was done with a total of 158 pediatric cases. Of these 158 cases, 98 cases were evaluated at post induction phase. MRD positivity at post induction was ~31.6% (31/98 cases) with a significant percentage of MRD positivity ~ 10.2% with MRD levels < 0.01% (10/98 cases). Out of 31 Post induction MRD positive cases, Post consolidation MRD was available for 22 patients. 06 out of these 22 cases (~27.2%) persisted to be MRD positive. All these cases were originally stratified as high risk and 03 of these 06 patients expired due to relapse. 16 out of 22 cases (~72.8%) reported to be post induction MRD positive became negative at post consolidation including all patients whose MRD levels were < 0.01%.

Summary and Conclusion: A total of 162 MRD positive cases (~30.79%) with MRD levels ranging from 0.0006% to 94% were identified. High risk patients had higher MRD positive rate in comparison to SR/IR stratified cases. 09 out of 98 cases (~9.18%) were upgraded from standard/intermediate risk to high risk arm. Out of the total Post Induction MRD cases where Post Consolidation phase MRD re-evaluation was done, ~27% persisted to be MRD positive even after consolidation. All these cases were stratified as high risk at diagnosis.

To conclude, MRD assessment is proven to have an important role for evaluation of initial treatment response and MRD based risk stratification. Hemodilution of sample often affects the sensitivity of the MRD assay and sending the first pull bone marrow sample for MRD analysis is a vital step for improving the sensitivity of the MRD assay.

PPCS-29

TITLE: Methotrexate treatment is associated with reduction of neutrophil reactive oxygen species and CD177 in RA patients.

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Objective: To study the effect of methotrexate (MTX) on neutrophils ROS production and activation markers in rheumatoid arthritis patients.

Methods: This was a cross-sectional study, on patients with rheumatoid arthritis (RA) and healthy controls. RA patients were grouped as naive-RA (on low dose steroids) and MTX-RA treated patients (at least 15 mg/week for 6 months). Neutrophils were isolated from blood using density-gradient centrifugation. Reactive oxygen species (ROS) by dihydrorhodamine (DHR) was detected using flow cytometry after PMA stimulation (1.62ug/ml). ROS by Luminol was detected by PMA (0.4ug/ml) and Luminol (50uM) using a luminescence detector (area under curve over 30 mins). Activation markers CD177,CD11b and CD64 were detected by surface staining of neutrophils using FACS.

Results: This study included 53 (F:M=43:10) patients of MTX-RA, 47 (F:M=39:8) naive-RA and 21 healthy controls. No significant difference in the mean age (44.5,47.6years,p=0.17) and a significant difference in the DAS28-3 seen between RA groups (6.2,4.9,p<0.001). At baseline, a higher level of ROS seen by DHR in naive-RA vs MTX-RA (11049, 7301, p=0.009) and remained higher in naive-RA than MTX-RA (88805**,**59637,p=0.0070) even after PMA stimulation. However, no significant difference seen between MTX-RA and healthy controls after PMA stimulation. No significant difference seen in ROS by luminol between naive-RA and MTX-RA patients. CD177 was highly expressed in naive-RA vs MTX-RA (46620,34475,p=0.0216) and healthy controls (46620,26855,p=0.0012). No significant change observed for CD11b and CD64 levels between RA groups. However, in comparison to healthy controls, MTX-RA patients had lower levels of CD64 (260,241,p=0.0115) while both MTX-RA and naive-RA patients had higher levels of CD11b (3708,5560,p=0.0112 and 3708,5978,p=0.0165).

Conclusion: MTX treatment in RA patients was associated with reduction of ROS production in neutrophils. Furthermore, CD177 expression was also significantly reduced in MTX- treated patients. One of the ways MTX acts in RA may be through reducing neutrophil activation and ROS.

PPCS-30

TITLE: **Flowcytometry in chronic T-cell lymphoproliferative disorders : study from a tertiary oncology centre in South India.**

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Objectives: To study the morphologic and immunophenotypic profile of T-cell chronic lymphoproliferative disorder (CLPD) by flowcytometry and to determine the incidence of various subtypes.

Materials and methods: All consecutively diagnosed cases of chronic T-cell lymphoproliferative disorders diagnosed by flowcytometry from 1st August 2015 to 31st July 2019 were retrospectively analysed and studied. Diagnosis of CLPD was made based on peripheral blood counts, examination of peripheral smear & bone marrow aspirate and immunophenotyping by flowcytometry. A panel of antibodies comprising of CD2, CD3, CD7, CD4 , CD8 , CD25, CD5 , CD19, CD20 , CD34,CD 56, TCR αβ, TCR γδ and CD45 were used.

Results: Over a period of 4yrs, 321 cases were diagnosed as chronic lymphoproliferative disorder by flowcytometry, of which 291(91%) cases were B-CLPD and 30 (9%) cases were T-CLPD. Age ranged from 26 to 84 years with a median age of 55years. The male to female ratio was 2:3. Subtypes of T-CLPD include 19 cases (63%) of Adult T-cell leukemia/lymphoma (ATLL), 4 cases (13%) of sezary syndrome, 3 cases (10%) of T-cell large granular lymphocytic leukemia (T-LGL) , 2 cases (7%) of T-cell prolymphocytic leukemia (T-PLL) and 2 cases (7%) of hepatosplenic T cell lymphoma(HSTL). All cases of ATLL showed CD4+/CD8-/CD7-/CD25+ phenotype. Loss of CD 5 expression was seen in 2 cases of ATLL. T-LGL in our series which revealed CD4-/CD8+ phenotype. Both cases of T-PLL were of CD4+/CD8- phenotype . Both cases of HSTL were CD 5-/CD7+. One case was CD4 -/CD8- where as the other one was CD4-/CD8+.

Conclusion: ATLL constitutes the predominant subtype of T-CLPD in our study.

PPCS-31

TITLE: **A small case series of flowcytometric analysis on fine needle aspirates of non lymphoid region swellings presenting with a cytomorphological differential diagnosis of Haematolymphoid neoplasm**

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Objective: Flowcytometric immunophenotyping (FCI) is an essential ancillary technique for classifying haematolymphoid neoplasm (HLN). These malignancies may come as a differential diagnosis in aspirates from nonlymphoid tissues where they may turn out to be an HLN or may turn out to be other kind of small round cell tumour. The objective of the study was to determine the utility of flowcytometric analysis on fine needle aspirates of non lymphoid region swellings presenting with a cytomorphological differential diagnosis of Haematolymphoid neoplasm

Materials and methods: Flowcytometric analysis was carried out on 3 laser, 8 color, 10 parameters BD FACS Canto II flowcytometer. For analysis FACS DIVA 8.1 version software was used.The inclusion criteria were cases on which on cytology smear there was a differential diagnosis of HLN. The fine needle aspirates were collected directly into EDTA vial containing sheath fluid (isotonic fluid). A cell count was taken on cell counter on fluid mode. Depending upon the concentration of cells the amount of sample was used. The stain-lyse-wash protocol was used.

Observation and results: The anatomical regions' fine needle aspirates that presented with a cytomorphological differential diagnosis of HLN were maxillary region swellings (2/7), thyroid swelling (1/7), breast lump (1/7), mandibular swelling(1/7), one case of malignancy floor of mouth (1/7), one case of surgical scar site swelling near medial canthus (1/7) . On a preliminary CD45, SSC, CD138 analysis four out of seven (57%) cases turned out to be non-haematolymphoid with negative CD45. On further cytohistological correlation, these cases were medullary carcinoma of thyroid, breast carcinoma with small cell morphology, small cell carcinoma nasal region, small cell variant of squamous cell carcinoma floor of mouth. The three cases where HLN was suspected on a preliminary diagnosis, complete panel was applied and a final diagnosis of Burkitt Lymphoma of the jaw, one case each of myeloid sarcoma and high-grade B cell lymphoma of maxillary region came out.

Conclusion: The cases in which on cytology there is a differential diagnosis of HLN, a preliminary tube can help in solving the dilemma. And a further complete panel can be applied afterwards in which the preliminary tube indicates towards a hematolymphoid malignancy.

PPCS-32

TITLE: Co-expression of strong CD38 and homogenous CD10 is a highly useful feature to identify Burkitt lymphoma using multicolor flow cytometry

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Objective: Burkitt lymphoma (BL) is an aggressive B-NHL which requires correct, urgent diagnosis. The characteristic morphological features, immunohistochemical expression of BCL2(-), BCL6(+), CD10(+) and 100% MIB-1(+) greatly aid in the diagnosis of BL. However, these features may overlap with other CD10+ B-NHL like diffuse large B-cell lymphoma (DLBCL) or follicular lymphoma transforming to DLBCL making the final diagnosis challenging. Molecular genetic studies for c-MYC, BCL2, and/or BCL6 rearrangements play a crucial role in further categorization. Unfortunately, these tests are time-consuming, relatively expensive and not routinely available at many laboratories, requiring submission to a reference laboratory, which can delay accurate diagnosis.Flow cytometric immunophenotyping (FCI) is a widely available, fast and inexpensive technique that routinely uses CD10 and CD38 for the diagnosis and monitoring of hematological neoplasms. We investigated the utility of CD10 and CD38 in the rapid diagnosis of BL.

Methods: We analyzed expression of CD10 (APC, clone-ALB1) and CD38 (BV605, clone-HB7) in CD10+ B-NHL. The final diagnosis was performed on histopathological and immunohistochemical evaluation. Cytogenetic findings were included wherever available. FCI was performed on Cytoflexflow-cytometer and data-analysis was performed using Kaluza-v2.1-software. Expression-levels of CD10 and CD38 were determined as a normalized mean fluorescent intensity (nMFI) as described by Sędek*et al.*

Results: We studied 36 cases of CD10+ B-cell NHL (16 BL; age, 4-67 years and 20 non-Burkitt NHL; age, 31-78 years). Non-Burkitt NHL included 15 follicular lymphomas (FL) and 5 GCB-DLBCLs. Median (range) of nMFI of CD10 and CD38 in BL, FL and GCB-DLBCL were 8.3 (0.05-75.9) and 8.71 (0.03-33.4), 9.95 (0.3-33.6) and 0.35 (0.008-6.8), 9.73 (2.1-19.3) and 0.6 (0.2-2.6) respectively. Strong expression was defined as nMFI>7. CD38 was strongly expressed in BL (13/16) versus non-Burkitt NHL (0/20) (median 8.71 vs 0.35, $p<0.001$). CD10 expression-level didn't reveal a significantdifference, but all 13/16 BL showed homogenous expression of CD10 versus 10/20 in non-BL. Co-expression of strong CD38 and homogenous CD10 was found in 12/16 BL but 0/20 non-BL NHL. Sensitivity and specificity of co-expression of strong CD38 and homogenous CD10 for the diagnosis BL was 100% and 83.3% ($p<0.001$) respectively. Cytogenetics was available in six BL cases and showed typical MYC:IGH translocation.

Conclusion: The co-expression of strong CD38 and homogenous CD10 is highly sensitive and specific in the differential diagnosis of BL from other CD10+ non-BL NHL.

PPCS-33

TITLE: Utility of FLAER and CD157 in a five-colour single tube combination, for diagnosis of Paroxysmal Nocturnal Hemoglobinuria – A stand-alone flow cytometry laboratory experience

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Objective: Paroxysmal Nocturnal Hemoglobinuria(PNH) is an acquired hematopoietic stem cell disorder caused by somatic mutation of PIG-A gene. PNH diagnosis using FLAER is the gold standard as per 2010 International guidelines. CD157 antibody is brightly expressed on both granulocytes and monocytes and has been found to have high sensitivity and specificity ascertaining as a new promising PNH marker.

Materials and methods: A total of 364 cases (Gender- M-192, F-172; Age Range– 4years- to 78years) with clinical suspicion of PNH were evaluated over a two year period. 100ul of EDTA whole blood was stained with a five-colour antibody cocktail consisting of FLAER-Alexa488/CD157PE/CD15PC5.5/CD64PE-Cy7 & CD45APCH7. Stain-Lyse-Wash technique was used for processing and samples were acquired on a BD FACSCanto-II instrument. A minimum of 50,000 neutrophils were acquired and if there were any positive events, reflexely 2,50,000 neutrophils were acquired for a high sensitivity assay (0.01%). A threshold cluster of 10-events was set for identifying a PNH clone.

Results: Of 364 cases, 59(16.2%) cases showed presence of a PNH clone in both neutrophils and monocytes whereas 305 cases lacked a PNH clone. PNH clone sizes ranged from 0.02% to 96.6% in granulocytes and 0.03% to 96.3% in monocytes based on their FLAER negative, CD157 negative phenotype. 22 of the 59 PNH-cases had WBC-clone size of less than 1%. Most common clinical indication amongst cases showing a PNH clone were aplastic anemia or pancytopenia. In addition, there were three cases where all neutrophils and monocytes were CD157 negative. Incidentally all three cases showed a PNH clone in both neutrophils and monocytes based on absence of FLAER expression and also showed a significant normal population which expressed FLAER but were CD157 negative (Figure1). Selected cases were rechecked with fresh cocktail and compared with control cases but showed consistent results indicating potential polymorphism in expression of CD157

Conclusion: The combination of FLAER and CD157 was successful for diagnosis of PNH with an incidence of 16.2% in our laboratory. We show three index cases with potential CD157 polymorphism which has not been described in literature before and re-highlight the importance of using FLAER as a gold standard for high sensitivity PNH assay.

PPCS-34

TITLE: Evaluation of surface molecule CD229 as an alternate marker of plasma cells on flow cytometry

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ObjectiveTo study and compare the surface expression of CD229 on Normal plasma cells (NPCs); other hematopoietic cells; abnormal plasma cells (APCs) of treatment naïve and patients of multiple myeloma (MM) on therapy.

Introduction: Multiparameter flow cytometric (MFC) characterization of plasma cells (PCs) is based on combination of CD38 vs CD138 vs CD45 with light scatter features [reference gating strategy (RG)]. However, detection of PCs by routine gating could be hampered due to various reasons such as processing and therapy related effects. Of all the new markers described in literature, we evaluated the expression profile of CD229 on PCs in view of its strong expression in MM cases.

Material & Methods: Bone marrow aspirate samples of MM (35 cases) and non-plasma cell neoplasms (20 cases) were acquired on BD FACS Canto II flow cytometer & data was analysed using Kaluza software for studying the expression-pattern of CD229. Two CD229 based gating strategies (CD229 vs. CD38 vs CD45; SG1) and (CD229 vs.CD138 vs CD45; SG2) were used & the mean total plasma cell percentage (TPC) were calculated and compared to the RG strategy.

Results: Out of 35 cases of MM, 22 newly-diagnosed and 13 patients on therapy (4 relapsed cases, 3 MRD positive and 6 MRD negative) median fluorescent intensity (MFI) of CD229 on NPCs and APCs was significantly higher than all the other hematopoietic cells (p value <0.001). There was marked downregulation CD38 expression in APCs compared to NPCs. However, CD229 expression was relatively stable in both NPCs & APCs. CD229 based gating strategies (SG1 and SG2) in the entire cohort showed significantly high correlation with the RG strategy.

Conclusion: The present study highlights that CD229 is a reliable & suitable surface marker for PCs & should be used in appropriate clinical scenario where an alternate to CD38 becomes necessary.

PPCS-35

TITLE: Role of Multiparametric Flowcytometry in predicting the probability of leukemic spill over of Myeloma

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Institution: Department of Pathology,[1] Department of Medicine[2]Maulana Azad Medical College, New Delhi

Objective: Plasma cell leukemia(PCL) is leukemic state of Myeloma. This could be primary(1º) or secondary(2º) where the circulating clonal plasma cells(PCs) in blood are >2000/µL or 20% of all nucleated cells. Myeloma cells spill over to blood is hypothesized to be due to reduced expression of the cell adhesion molecule CD56,overexpression of CD27and loss of CD81.Along with CD19,CD20 and CD28 positivity, this phenomenon is often observed among patients with 1ºPCL,while the positive expression of CD28, interleukin-6 and expression of CD27 after bortezomib treatment are considered important in 2ºPCL.Leukocytosis,high levels of LDH and β2-microglobulins &the presence of bone lesions can help to differentiate 1ºPCL from myeloma.

Method: This study was conducted in department of Pathology,MAMC&paired samples of blood along with BM were taken and flowcytometry was performed synchronously at the diagnosis.5/6 cases had PCs in blood&BM, with PCs count ranging from 0-23% in blood and 25-90% in BMA. Multiparameter flowcytometer five colour with three lasers(Beckman coulter),using following panel:CD45,CD19,CD20,K,λ,CD56,CD38,138,27,28,200,117&81

Result: Expression of CD56,CD117andCD27 was found to be positive in 4/6,1/6 and3/6 cases in blood while one case had dim positivity of CD19 in blood and BMA.One case had expression of CD200,28&81 in blood & loss of CD28 in BM.

Conclusion: This study proposes the utility of doing synchronous analysis of paired sample of blood and BM in multiple myeloma cases to predict the probability of circulating PCs in blood and thus PCL as the latter has poor prognosis and needs correct sub-categorization.

PPCS-36

TITLE: Biclonal B-Chronic Lymphocytic Leukemia

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Objectives: Chronic Lymphocytic Leukemia/Small Lymphocytic Leukemia is a neoplasm composed of monomorphic small mature B cells that co-express CD5 and CD23. However, CLL with two populations of B cells expressingboth kappa as well as lambda (biclonal) light chains are extremely rare. Immunophenotyping has become an integral part of the classificationof the chronic B-cell leukemias and often is considered essential in the initial diagnosis. We report a case of Biclonal B-Chronic Lymphocytic Leukemia.

Material and Method: A 65year female presented to the hospital with complaints of generalized weakness, fever and pain lower limbs of 2-3months duration. Haemogram revealed Hb 11.5g/dl, WBC 85400/uL, platelet count 182000/uL. Peripheral smear reveal smudge cells. Hence, the possibility of chronic lymphoproliferative disorder was made. Flowcytometric analysis of peripheral blood revealed 69.7% B-lymphoid cells which revealed expression of B-cell markers- CD19, CD20, CD22 with co-expression of CD5 and CD19, co-expression of CD5 and CD23. On evaluation of surface light chains, a major subset of atypical lymphoid cells was positive for kappa, and other subset was positive for lambda surface light chain. This biclonality is specific because of applied gating was sequential. Therefore, in view of immunophenotypic profile, an unusual diagnosis of Biclonal B-Chronic Lymphocytic Leukemia was made.

Result and Conclusion: Multiparameter flow cytometry helped in identification of the rare case of biclonal disease in chronic lymphocytic leukaemia (CLL). Mere calculation of light chain ratios can be misleading. Hence, this case highlights the importance of morphology of the blood smear and the entire immunophenotype panel to arrive at a precise diagnosis.

PPCS-37

TITLE: Mixed phenotype acute leukemia (T/Myeloid) arising in the background of Juvenile myelomonocytic leukemia in a 3 year old child; A Rare Transformation

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Objective: Juvenile myelomonocytic leukemia (JMML) is a typically aggressive myeloid neoplasm of childhood that is clinically characterized by excessive proliferation of cells of monocytic and granulocytic lineages. JMML is categorized as an overlap myelodysplastic syndrome/myeloproliferative neoplasm (MDS/MPN) by the World Health Organization and is characterized by peripheral blood monocytosis>1000/cmm and <20% blasts in the bone marrow. Several congenital syndromes predispose to JMML, including neurofibromatosis type 1 (NF1) and CBL syndrome. Genetic mutations in NF1, NRAS, KRAS, RRAS, RRAS2, PTPN11, and CBL are all hypothesized to increase signalling through the Ras/MAPK pathway, which is a cardinal molecular feature that can be identified in approximately 95 percent of patients. Blastic transformation is infrequent in JMML and most of them progress to acute myeloid leukemia, however progression to mixed phenotype acute leukemia (MPAL) is even rarer. Here, we report a rare case of JMML in a 3 year old child that transformed to MPAL with T/Myeloid immunophenotype.

Case Summary: A 3 year old male presented with fever for 1 month along with abdominal swelling and petechial rash. On examination, he was found to have hepatosplenomegaly and subcentimeter cervical lymph nodes. His Hb was 7.7g/dl, TLC 16700/cmm, TPC 58000/cmm with absolute monocyte count of 1420/cmm. Bone marrow examination shows a dilute yet cellular bone marrow with myeloid preponderance and 16% blast/hematogones like cells with no definite dyspoiesis. Immunophenotypic analysis revealed 3.3% abnormal myeloid blasts along with 18% hematogones and next generation sequencing assay revealed a somatic PTPN11 mutation. A diagnosis of JMML was made and he was started on OMCT (MP, Etoposide, Prednisolone) regimen. A follow up peripheral blood immunophenotyping after 8 months shows presence of 4.2% abnormal myeloid blasts, however a small subset (8.8%) of blasts expressed cytoCD3. Repeat bone marrow examination was done after 3 months which revealed mildly hypercellular marrow with 27% cytochemical MPO negative blasts expressing a mixed immunophenotype of both T cell & myeloid lineage on flow cytometry. So a diagnosis of JMML transforming into MPAL was given and referred for bone marrow transplant.

Conclusion: MPAL arising in the background of JMML is extremely uncommon and has dismal outcome. Additional de novo or therapy related molecular abnormalities may play in pathophysiology of these malignancies. Immunophenotyping and molecular studies are important for diagnostic as well as therapeutic purpose in such aggressive neoplasms.

PPCS-38

TITLE: Utility of single tube 16c antibody panel for detection of minimal residual disease of unusual immunophenotype in a case of acute myeloid leukemia in a 4 year old male

Devasis Panda, Gaurav Chatterjee, Sitaram Ghoghale, Pratyusha Gudapati, Nilesh Deshpande, Badrinath Y, Nikhil Patkar, BhausahebBagal, Hasmukh Jain, Manju Sengar, NavinKhattry, Subramanian PG, Sumeet Gujral, Prashant Tembhare

Institution: Tata Memorial hospital, Mumbai

Objective: Minimal residual disease (MRD) is defined as post therapy persistence of leukemic cells at levels below morphological detection which is an independent prognostic marker of increased risk of relapse and shorter survival in patients with acute myeloid leukemia(AML). Detection of MRD by multiparameter flow cytometry(MFC) is achieved by the identification of immunophenotypic aberrancies on myeloblasts and/or monoblasts/promonocytes. 10-color MFC has been shown to reliably detect MRD with a sensitivity of approximately 0.01%. However, occurrence of rare immunophenotype in abnormal blasts warrants combination of increased number of antibodies for MRD detection. Here we report the utility of a single tube 16c antibody panel for detection of such rare MRD in a 4-year old child diagnosed with AML.

Materials and Methods: A 4-year boy diagnosed case of AML elsewhere came for evaluation of post induction MRD. Wright stained bone marrow aspirate smears showed a dilute marrow with 3% blasts. Bone marrow aspirate sample was processed by bulk-lyse-stain-wash technique and a single tube 16-color antibody panel was used. Data was acquired on BD-LSR fortessa instrument and was analysed in Kaluza-2.1 software.

Result: Immunophenotypic analysis revealed 1.4% MRD which expressed dim CD45, bright HLA DR, moderate CD33, moderate CD123, moderate CD36, dim-negative CD15 and are negative for CD34, CD117, CD14, CD64, CD38, CD19, CD13, CD7, CD56 & CD90. In the absence of diagnostic phenotype, we could identify such rare immunophenotypic abnormality with the help of 16-color combination of antibody panel.

Conclusion: Single tube 16-color antibody panel provides more detailed immunophenotypic analysis to pick up unusual aberrancies compared to 10-color antibody panel. It also requires less sample and can be incorporated in MRD panel in such difficult cases.

PPCS- 39

TITLE: Minimal residual disease assessment in a case of lymphoplasmacytic lymphoma in a 27year old male: Adding a new entity to MRD detection

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Institution: Tata Memorial hospital, Mumbai

Objective: Minimal residual disease (MRD) is defined as post therapy persistence of leukemic cells at levels below morphological detection which is an independent prognostic marker of increased risk of relapse in patients with acute leukemias. Detection of MRD by multiparameter flow cytometry(MFC) is achieved by the identification of immunophenotypic aberrancies on leukemic cells. However, MRD evaluation in cases of lymphoproliferative disorders is not often followed in routine clinical practice. Here we report a case of lymphoplasmacytic lymphoma in a 27-year-old male which came to us after 4 cycles of BRD regimen for remission status.

Materials and Methods: A 27-year-old male diagnosed case of lymphoplasmacytic lymphoma came for evaluation of MRD after 4 cycles of BRD regimen. Wright stained bone marrow aspirate smears showed a dilute yet cellular marrow with no obvious morphological evidence of abnormal lymphocytes. Bone marrow aspirate sample was processed by bulk-lyse-stain-wash technique. A routine single tube 12-color antibody panel was initially used followed by a custom antibody combination in reference to the diagnostic immunophenotype. Data was acquired on Beckmann-Coulter Cytoflex instrument and was analysed in Kaluza-2.1 software.

Result: Immunophenotypic analysis revealed 0.01% MRD which expressed the same immunophenotypic aberrancies as that of the diagnostic one. In view of the young age of the patient, a conservative treatment approach could be followed simultaneously with the help of MRD monitoring.

Conclusion: MRD evaluation in cases of lymphoproliferative disorders is rarely followed. However, for newer treatment modalities, MRD evaluation may be incorporated into routine clinical practice to increase therapeutic efficacy.

PPCS-40

TITLE - Paroxysmal nocturnal hemoglobinuria detection by flow cytometry - a one year study at a tertiary care hospital

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Objective: Paroxysmal nocturnal hemoglobinuria (PNH) is a rare condition due to a nonmalignant clonal expansion of hematopoietic stem cells that have an acquired mutation in PIGA. It presents with hemolytic anemia, marrow failure and thrombophilia. PNH diagnosis by flow cytometry has now replaced earlier less sensitive tests such as Hams's test and Sucrose lysis test. The Objective of our study was to determine the incidence and clinical features of patients that demonstrated PNH clones from all cases screened by flow cytometry over a period of one year.

Materials and Methods: Our study was a retrospective analysis. 110 patients with either hemolytic anemia, cytopenia, myelodysplastic syndrome or thrombotic episodes were screened for the presence of PNH clones. Red blood cells were studied for expression of GPI-linked membrane proteins CD55 and CD59. Neutrophils were studied for expression of CD24 and Fluorescein-labeled proaerolysin (FLAER) while monocytes were studied for expression of CD14 and FLAER.

Results: From a total of 110 patients screened, 6 patients (5.4%) were positive for PNH clones. The PNH clone in neutrophils ranged from 1.2% to 82% and 1.5% to 90% in monocytes. The maximum clone percentage in RBCs was 15%. All six patients' cases of PNH were in the setting of another specific bone marrow disorder. Four patients had aplastic anemia and two patients had myelodysplastic syndrome. Two patients presented with features of hemolysis.

Conclusion: Of the 110 patients screened for PNH by flow cytometry at our centre over one year, 6 were positive for PNH clones. Although this incidence is quite low compared to other Indian studies it was higher compared to our earlier detection rate when using Ham's test (only 2 cases were positive over a period of 10 years). The usage of FLAER also increases the detection of small PNH clones.

PPCS-41

TITLE: Leucocyte Adhesion defeciency type 1 – An atypical clinical presentation compounded by a co-existing myeloperoxidase deficiency

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Objective: Leucocyte Adhesion deficiency (LAD) is a group of rare autosomal recessive disorders characterized by immune deficiency and peripheral blood neutrophilia. We report a case of LAD-1 who presented with unusual clinical features and presented a diagnostic challenge in view of a co-existing myeloperoxidase deficiency.

Materials and methods: A 1-year old girl who appeared small for age presented with history of repeated infections since the age of 2.5 months. She had history of scarring at intramuscular injection sites, ear discharge which cultured pseudomonas and repeated skin infections with peeling. She has an elder brother, 5 years old, who is healthy. There was no history of delayed cord fall at birth. The mother has a history of 2 previous miscarriages. Her initial WBC counts during the first presentation were 1.2lakh/ul and have now gradually reduced to 25,000/ul at current presentation. Sample was sent for Primary immunodeficiency panel (TBNK lymphocyte subsets, Dihydrorhodamine, Nitroblue tetrazolium and Immunoglobulin levels). There was a mild elevation in IgG and IgA, while IgM, IgE and lymphocyte subsets were within normal limits. Neutrophil respiratory burst by Dihydrorhodamine test was markedly reduced, while Nitroblue tetrazolium dye reduction was preserved. In view of the marked leukocytosis, a CD18 count was performed, which showed near complete absence of CD18 expression on neutrophils, indicating Leucocyte Adhesion Defect type 1. Myeloperoxidase cytochemistry confirmed myeloperoxidase deficiency, which explained the discrepant NBT and DHR results. Whole exome sequencing revealed two pathogenic mutations in the ITGB2 gene (c.817G>A and c.382G>A), along with a possibly pathogenic mutation in the SCN5A gene (c.2186 T>C). This child is currently undergoing a bone marrow transplant.

Conclusion: Myeloperoxidase deficiency often remains asymptomatic. However, awareness of it's impact on a dihydrorhodamine test is important to avoid a false positive diagnosis of chronic granulomatous disease.

PPCS-42

TITLE: Marked leucocytosis in young adult: an interesting case

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Institution: Departmentof Pathology, Dr Ram Manohar Lohia Institute of Medical Sciences

Objectives: T-cell Prolymphocytic leukaemia is a rare malignancy(2% of mature lymphocytic leukemias) characterized by the proliferation of small to medium sized prolymphocytes of postthymic origin with distinctive clinical, morphologic, immunophenotype, and cytogenetic features.

Materials and methods: A 34 yrs male was referred to Hematology lab for bone marrow examination with a provisional diagnosis of Acute leukemia. He presented with a history of axillary and cervical swelling, petechiae over the back and generalized weakness for 4 months. PBS showed presence of marked leuocytosis(TLC:76,000) 90% atypical cells. These atypical cells were small to medium sized with round oval to markedly irregular nuclei, condensed chromatin, visible nucleolus and agranular basophilic cytoplasm with cytoplasmic blebs. Bone marrow biopsy showed infiltration by similar cells. Excision biopsy of the cervical lymph node showed complete effacement of the architecture by a diffuse infiltrate of atypical lymphoid cells. Based on the FCM on blood, bone marrow examination and lymph node biopsy results diagnosis of T-NHL(Mature T cell type)was given. Patient was started on Cladribine and reviewed after 6 months. PBS showed 84% atypical lymphoid cells.

Results: The atypical cells were positive forCD3, CD4, CD8, CD5, CD7, CD26 and TCL-1 while negative for CD20, Tdt, CD1a, CD5, CD56 and TCRGD. A final diagnosis of T-Cell Prolymphocytic leukemia was made. Patient is presently clinically well, skin rashes have reduced and TLC is 43,000. He is being prepared for bone marrow transplant.

Conclusion: T-PLL are rare tumours particularly in young age. It is often misdiagnosed as acute leukemia or other subtypes of T-NHL. To discriminate between these malignancies, it is crucial to integrate all the clinical and laboratory information (PB morphology, histology, immunophenotyping and genetic markers). Co expression of CD4/CD8 is highly suggestive while TCL 1 expression is 100% specific and 70-80% sensitive. Owing to its aggressive and rapid clinical course, delay in making the diagnosis may lead to a fatal outcome. Patients who achieve a response to therapy are considered for stem cell transplantation (SCT).

PPCS-43

TITLE: Immunophenotypic aberrancies in acute lymphoblastic leukaemia : a study from a tertiary referral centre in south Tamil Nadu.

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Objectives: To enumerate the immunophenotypic aberrancies in acute lymphoblastic leukaemia.
Materials and Methods: All the cases newly diagnosed as Acute Lymphoblastic Leukaemia by flow cytometric analysis in our institute between December 2017 and July 2019 were included in this study and retrospectively analysed. Either peripheral blood or bone marrow samples were studied using BD FACS CANTO II (Diva software). Immunophenotypic analysis was performed in all cases based on a pre-defined diagnostic panel of antibodies. Among aberrant patterns, expression of myeloid antigens (CD33, CD13 & CD117) in ALL cases, T-cell antigen in B-ALL cases and B-cell antigen in T-ALL cases were evaluated.

Results: Out of 284 cases diagnosed as acute leukaemia, 152 (54%) were ALL. Among ALLs, 118 cases (78%) were B-ALL, 34 cases (22%) were T- ALL. 26 cases (17%) expressed at least one aberrant myeloid antigen. 16 cases (14%) of B-ALL and 10 cases (29%) of T-ALL expressed myeloid antigens. CD 33 was the most commonly expressed aberrant myeloid antigen. In B-ALL, 6 cases (5 %) were CD 13 positive, 13 cases (11%) CD 33 positive and 1 case (0.8%) CD117 positive. 4 cases were positive for both CD13 and CD33. One case each of aberrant expression of CD 5, CD7 and CD 4 (T cell antigens) was noted.In T-ALL, 3 cases (9%) were CD 13 positive, 6 cases (18%) CD33 positive and 2 cases (6%) CD117 positive.

Conclusion: CD 33 was the most common aberrant myeloid marker expressed by both B-ALL and T-ALL. Evaluation of immunophenotypic aberrancies remains valuable for a precise characterisation of the leukaemic population and for monitoring minimal residual disease.

PPCS-44

TITLE: **Rarest of the rare–mixed phenotype acute leukemia (MPAL), B/T with CD45 negative T-lymphoblasts**

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Presenting Author:
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Objective: Rare leukaemias (<4%) that do not show clear evidence of differentiation towards a single lineage, classified as Acute leukaemias of ambiguous lineage, need to be explored so that unusual variations can be known.

Materials and methods: Peripheral blood of a suspected case of Acute leukemia was analyzed by Multicolor Flow Cytometry (MFC) using 8-color panel with following antibodies in various combinations: CD45, CD22, CD34, CD19, CD10, CD38, CD5, CD3, CD4, CD7, CD64, CD13, HLADR, CD117, CD33, CD14, CD20, CD2, CD56, CD8, CD4, CD99, CD5, TCRγδ, CD1a, CD71, ANTI KAPPA, ANTI LAMBDA, CD19, cytoplasmic MPO, cytoplasmic CD79a, cytoplasmic TdT, cytoplasmic CD3. They were processed by Stain-Lyse-Wash method on BD FACS CANTO II and analyzed using BD FACS DIVA Software v8.0.1.

Case History: A 32 years old male presented with neck swelling and intermittent fever since 6 weeks. On examination, he was found to have bilateral cervical, axillary and inguinal lymphadenopathy and no hepatosplenomegaly. CBC showed a total WBC count of 112x109/L with 76% blasts on morphology. Hence, flow cytometry was performed to know the diagnosis.

Results: On CD45 vs Side Scatter (SSC) plot, abnormal populations were gated and studied. The first population (63.2% of all viable cells) showed moderate CD45 and low SSC expressing markers of B lineage with CD19, CD22, CD10, CD20, HLADR, CD38 and cytoplasmic TdT. The second population (14%) showed negative CD45 and low SSC T lymphoblasts which was unusual and rare, expressing markers of T lineage with CD3, CD7, CD5, CD4, CD8, CD2, CD38, CD99, cytoplasmic CD3 and cytoplasmic TdT. This satisfies both WHO 2017 as well as EGIL 2015 criteria for classifying the case as Mixed phenotype acute leukaemia, B/T.

Conclusion: Flow cytometric analysis of every cluster is essential along with knowledge of rare variations in order to arrive at an accurate diagnosis.

PPCS – 45

TITLE: Mixed phenotype blast crisis in a case of Chronic Myeloid Leukemia

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Objective: CML Blast crisis is characterized by either ≥20% blast cells in the peripheral blood smear or in the bone marrow, or an extramedullary accumulation of blast cells, or large foci or clusters of blasts in the bone marrow biopsy.The phenotypic expression by blasts is reported to be usually of myeloid lineage (70%) and lymphoid lineage (30%). Mixed phenotype blast crisis in CML is reported rarely.

Case Presentation: A 56-year-old female presented with generalised weakness and fever for 3 months. On examination she had pallor and moderate splenomegaly.CBC showed microcytic hypochromic anaemia (Hb4.9gm/dl),thrombocytopenia(31x109/L),leucocytosis(70x109/l).Morphological examination of slide showed immature granulocytic precursors(Metamyelocyte04%,Myelocyte19%),pleomorphic blast cells(12%)along with Neutrohili31%,Lymphocytes30%,Eosinophil01%,Basophil01%.A provisional diagnosis of Myeloproliferative neoplasm was made. Bone marrow was examined. The smears were hypercellular and showed reduced erythropoiesis and megakaryopoiesis. Blast constitute 30%,suggesting blast phase conversion in MPN. Conventional cytogenetics was positive for t(9;22).Flow cytometry (4 color ,BD FACS Caliber)revealed approx 46%,blasts in dim CD45 region.The blast were bright positive for CD34,TdT,HLA DR,cCD79a,cCD22 and cMPO. Moderate expression was seen for CD19 and CD10.CD13 and CD33 were dim. Blasts were negative for CD2,CD7,CD3,cCD3,CD25,CD20 and CD64.

Conclusion: Blast crisis with mixed phenotype in CML is rare.In our records out of 20 diagnosed cases of blast crisis(60%myeloid and 40%lymphoid) in last 2.5 years this is the first case.Mixed phenotype blast crisis is associated with poor outcome.

PPCS-46

TITLE:-Angioimmunoblastic T-cell lymphoma diagnosed on flowcytometry

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Objectives: Angioimmunoblastic T-cell Lymphoma is a neoplasm of mature TFH cells characterized by systemic disease. It is a rare form of Peripheral T-cell Lymphoma representing only 1 to 2% of all cases of Non Hodgkin's lymphoma and nearly 1 in 5 cases of Peripheral T-cell lymphoma diagnosed per annum. Its diagnosis remains a challenge as clinical presentation as well as pathological findings are frequently misleading. We present a case of Angioimmunoblastic T-cell Lymphoma diagnosed on FNAC Flowcytometry.

Materials and Methods: A 64-year-old male presented with bilateral cervical, axillary and intra abdominal lymphadenopathy with splenomegaly 3 cm below costal margin. His CBC parameters were Hb: 8 gm%, TLC: 6500/cu.mm, DLC: P60, L30, E4, M6, Platelet: 2,10,000/cu.mm. Biochemical parameters were Serum Proteins: 11.6 g/dl, Albumin 4.5 g/dl, Globulin: 7.1 g/dl, LDH: 600U/L. FNAC done from cervical node was suggestive of Non Hodgkin's Lymphoma. Flowcytometric immunophenotyping was done from fine needle aspirate sample using BD FACS Calibur.

Results: Cells were gated on CD45 versus side scatter. Approx 60% cells gated were found to be positive for CD3, CD5, CD4, CD10 and negative for CD20, CD8, CD19, CD34. So diagnosis of T cell lymphoma with TFH cell origin was made. Meanwhile we also received biopsy of lymph node. Histomorphology showed effaced architecture with partially preserved follicles. Stroma has numerous high endothelial venules. IHC showed neoplastic cells positive for CD3, CD5, CD10 and BCL6. CD20, CD30, CD56, BCL2 were negative. So diagnosis of Angioimmunoblastic T cell lymphoma was made.

Conclusion: Angioimmunoblastic T-cell Lymphoma is a systemic disease characterized by monoclonal proliferation of T cells expressing CD3 and CD4. Characteristically, the tumor cells show immunophenotype of normal TFH cells, expressing CD10, CXCL13, BCL6. Flowcytometry on fine needle aspirate material helps in providing prompt diagnosis.

PCS- 47

TITLE: Simultaneous occurrence of CD20+/- lymphoma - a rare combination.

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Institution :[1]Department of Pathology, BMCHRC, [2]Senior consultant,Department of Medical Oncology, BMCHRC, [3]DNB resident,Department of Pathology, BMCHRC, Jaipur.

Objective: Composite lymphoma is a rare disease that has been defined in recent literature With the usefulness of multiparametric flowcytometric Immunophenotyping. This phenomenon get further rarest when the combination of CD20+ with CD20- Lymphoma occur at the time of initial presentation.CD20 loss in the B cell lymphoma is a well known phenomena after Rituximab therapy. However de-novo loss of CD20 is one of the rarest phenomena.

Material & methods: 58 year, old male came to our institute & found to have incidentally increased TLC count with Hb-5.6g/dl, platelet count-33000/cumm ,spleen-5cm, no Lymphadenopathy, coomb's (direct+ indirect)-negative, PBF-predominantly small size atypical lymphoid cells. Patient is HIV –ve. Flowcytometry was performed which shows 75% B cell population which is positive for CD20,CD22,CD79b,HLA-DR,IgM & show LAMBDA restriction with the subset of around 10% population which shows CD10,CD19,CD38,CD43 positive but clear cut CD20 negative. Then patient was put on Rituximab therapy, after 1 month decrease in TLC count with increase in medium to large size lymphoid cell was seen which was phenotyped& shows dominant clone of CD10+,CD19+,CD38+,CD20- & dim CD45+.Marker of immaturity were absent with persistence of surface immunoglobulin.

Result: This case highlights synchronous presence of two clones, one is CD20 positive, other is CD20 negative .CD 20 negative clone become prominent after 1 month of Rituximab therapy.

Conclusion: Composite Lymphoma must continue to be recognized because the disease subset may have variable natural histories, prognosis and different treatment modalities. Also study of such cases may provide us with the etiology & inter-relationship of clonal evaluation in lymphoma.

PCS-48

TITLE: Hairy cell leukemia without splenomegaly: a rare presentation.

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OBJECTIVE: Hairy cell leukemia(HCL) has a characteristic clinical, morphological and immunophenotyping profile is important to distinguish HCL from other B cell lymphoproliferative disorder due to availability of different chemotherapeutic agents.We present a case of HCL with only weakness but no typical symptoms such as splenomegaly and bone marrow fibrosis.

MATERIAL AND METHODS: A 43 years old man presented with generalized weakness. On examination there was no hepato-splenomegaly or lymphadenopathy. The initial complete blood count (CBC) revealed a hemoglobin concentration of 14.3 g/dL, white blood cell count of 4800 cells/cu mm, platelet count of 90,000 cells/cu mm. Bone marrow aspiration (BMA) and biopsy (BMB) was done. Immunophenotyping was done by flow cytometry (FCM).

RESULT:CBC showed thrombocytopenia only while peripheral blood smear examination revealed presence of occasional (8%) atypical lymphoid cells. BMA smears were cellular with adequate megakaryocytes and presence of 55% are atypical lymphoid cells of medium to large size with round to irregular nucleus, moderately fine chromatin and moderate amount of cytoplasm without hairy projection. Trephine biopsy revealed the typical fried egg appearance of hairy cells with no increased fibrosis. Immunophenotyping revealed the abnormal cell population expressing bright positivity for CD19, CD20, FMC7, CD200 with kappa chain restriction and dim positivity for CD25, CD103, CD23.

CONCLUSION:Hairy cell leukemia has classical traid of pancytopenia, splenomegaly and presence of hairy cells. Any deviation from these classical findings poses difficulty in diagnosis. This case was worth presenting because of absence of pancytopenia, palpable spleen and typical hairy cell. Careful morphological, immunophenotyping evaluation will helps in rendering correct diagnosis.

PPCS-49

TITLE: Detection of HLA-B27 by Flowcytometry in patients with suspected ankylosing spondylitis in Tertiary Care Centre

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Institution: SRM, Chennai

Objective: Human leukocyte antigen (HLA-B27) is strongly implicated in the pathogenesis of Ankylosing Spondilytis (AS). Hence, HLA-B27 testing is routinely used in the diagnosis of AS. The objective of the study was to establish the frequency of HLA-B27 in AS patients by flow cytometry and relate the differences between B27+ and B27- cases to the serum concentrations of rheumatoid factor (RA), erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP).

Methods: Study population included a total of 210 patients, who visited the Tertiary health care centre. The peripheral blood samples obtained from AS patients were subjected to a qualitative two-color direct immunofluorescence method using HLA-B27/CD3 antibody for the rapid detection of HLA-B27 antigen expression in erythrocyte-lysed whole blood in FACSCaliburflowcytometer (Becton Dinkinson, USA).

Results: Out of 210 AS patients, the distribution of HLA-B27 positivity was observed only in 46 (22%) patients. The remaining 164 patients (78.1%) were negative for HLA B27. Of the 46 HLA positive patients, 39 (25.34%) were males and only 7 (12.5%) were females. In both sexes, HLA B27 frequency was significantly higher in the age group 21-30 years, followed by 41-50 years. The current study also revealed a significant association between sex and age of onset of HLA-B27 detection in patients with suspected AS. Disease activity was not significantly correlated with RF, ESR and CRP.

Conclusions The detection of HLA-B27 by flow cytometry proved to be a reliable test in the screening of AS in Indian population.

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