

MOVING BEYOND THE ABC OF CBC

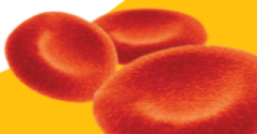
-CBC ADVANCE-

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drkunalsehgal@gmail.com



Moving Beyond the ABC of CBC

Anemia Diagnosis

<https://sehgalpathlab.com/>

<http://bit.ly/SehgalPathLab>

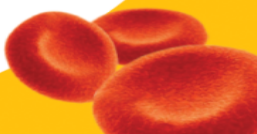


<http://bit.ly/SehgalPathLab>

TALK OVERVIEW

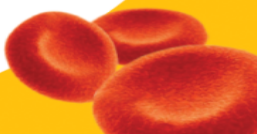
CASE BASED APPROACH TO DISCUSS THE FOLLOWING TOPICS –

- ANEMIA – DIAGNOSIS – Common Case studies
- ANEMIA OF CHRONIC DISEASE – CKD PATIENTS
- Reticulocyte count and its use in anemia
- Oncology Case studies



Getting more mileage out of CBC

- Basic RBC parameters and Graphs
- Instrument Flags
- Reticulocyte Mode based parameters

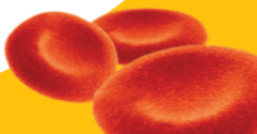


CBC ADVANCE

- CBC and Peripheral Smear
- Automated Beta Thalassemia Screening
- Automated Malaria Detection
- Automated Reticulocyte Count
- Reticulocyte Production Index
- Advanced CBC parameters - Ret He, IPF & others
- SIX part WBC DIFFERENTIAL with Immature Granulocyte Flagging



Sysmex XN-L 350



CBC ADVANCE Report

CBC ADVANCE HEMATOLOGY COMPLETE BLOOD COUNT (HAEMOGRAM)

<u>TEST</u>	<u>OBSERVED VALUE</u>	<u>REFERENCE RANGE</u>
Haemoglobin	7.8	13 - 18 gm/dL
R.B.C. Count	2.96	4.5 - 6.5 mill/cu.mm
PCV	23.8	40 - 54 %
MCV	80.41	76 - 96 fL
MCH	26.35	27 - 32 pg
MCHC	32.77	30 - 36 %
RDW	16.8	11.5 - 15 %
Total W.B.C Count	3490	4000 - 11000 /cmm

DIFFERENTIAL COUNT

Neutrophils	60	40 - 75 %
Lymphocytes	22	20 - 45 %
Eosinophils	06	1 - 6 %
Monocytes	11	2 - 10 %
Basophils	01	0 - 1 %

PERIPHERAL BLOOD SMEAR

RBC Morphology	Normocytic Normochromic with few microcytic hypochromic cells	
WBC Morphology	Mild Leucopenia	
Platelet Count	65000	150000 -400000 /cumm
Platelet On Smear	Platelets are reduced on smear with many giant platelets.	

*** END OF REPORT ***

CBC ADVANCE Report

<u>TEST</u>	<u>CBC ADVANCE HEMATOLOGY</u> <u>OBSERVED VALUE</u>	<u>REFERENCE RANGE</u>
Reticulocyte Count	3.30	0.42 - 1.82 %
RPI (Reticulocyte Production Index)	0.8	
Comments : RPI = Corrected reticulocyte count/ Reticulocyte maturation time in days RPI should be used only for adult anemic patients. RPI >2 indicates significantly increased hematopoiesis whereas RPI <2 indicates reduced response in an anemic patient.		
IRF (Immature Reticulocyte Fraction)	21.8	2.00 - 16.50 %
Comments : IRF gives an idea about the least mature erythrocytes which contain the most RNA. In many clinical situations, IRF increases before the total reticulocyte count and can be used to monitor BM response.		
Ret He (Reticulocyte Hemoglobin Equivalent)	23.8	28.7 - 34.1 pg
Comments : Ret He provides an indirect measure of functional iron over the last 3-4 days and is reduced in patients with functional iron deficiency. FID occurs when reticulo-endothelial stores are normal to high but iron is not delivered for erythropoiesis (eg. Chronic renal dialysis, Chronic inflammation, Cancer patients)		
IPF (Immature Platelet Fraction)	15.3	0.70 - 4.30 %
Comments : IPF (Platelet Reticulocyte count) is raised in patients with peripheral consumption/destruction of platelets (eg. ITP/TTP) and is normal or low in patients with BM failure. IPF can be used for predicting platelet count recovery post chemotherapy, stem cell transplant and dengue patients.		

*** END OF REPORT ***

Case 1

IPU - [Data Browser] Ver.:00-21 User Name:0

File(F) Edit(E) View(V) Record(R) Action(A) Report(P) Setting(S) Window(W) Help(H)

F1 Help F2 MANUAL F4 Menu F5 QC Files F6 Work list F7 Explorer F8 Browser F11 Validate F12 Out Upper Lower Last20 Delete

Positive No. 13
Action
Validated

16/05/2016 10:54:55 ID 50(Age) Female

Main Graph Cumulative Q-Flags Service Research

Items

Item	Data	Unit	LL	UL
WBC	7.04	10 ³ /uL		
RBC	4.88 *	10 ⁶ /uL		
HGB	11.0	g/dL		
HCT	38.4	%		
MCV	78.7 *	fL		
MCH	22.5 *	pg		
MCHC	28.6 *	g/dL		
PLT	365 *	10 ³ /uL		
RDW-SD	----	fL		
RDW-CV	----	%		
PDW	14.3 *	fL		
MPV	10.8 *	fL		
P-LCR	32.2 *	%		
PCT	0.40 *	%		

WBC Differential

Item	Data	Unit	LL	UL
NEUT#	4.91	10 ³ /uL		
LYMPH#	1.78	10 ³ /uL		
MONO#	0.22	10 ³ /uL		
EO#	0.10	10 ³ /uL		
BASO#	0.03	10 ³ /uL		

Item	Data	Unit	LL	UL
NEUT%	69.8	%		
LYMPH%	25.3	%		
MONO%	3.1	%		
EO%	1.4	%		
BASO%	0.4	%		

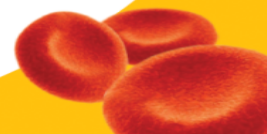
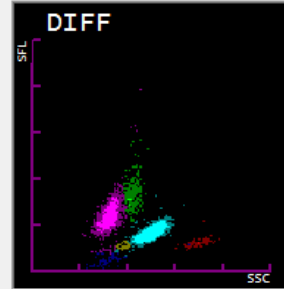
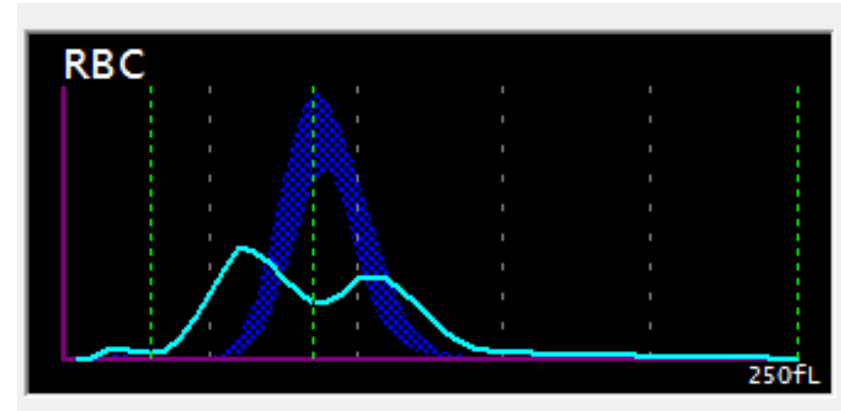
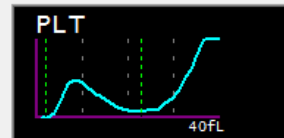
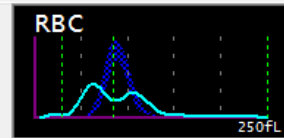
Flag(s)

WBC RBC PLT

RBC Abn Dst
Dimorph Pop
Hypochromia
Anemia

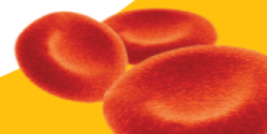
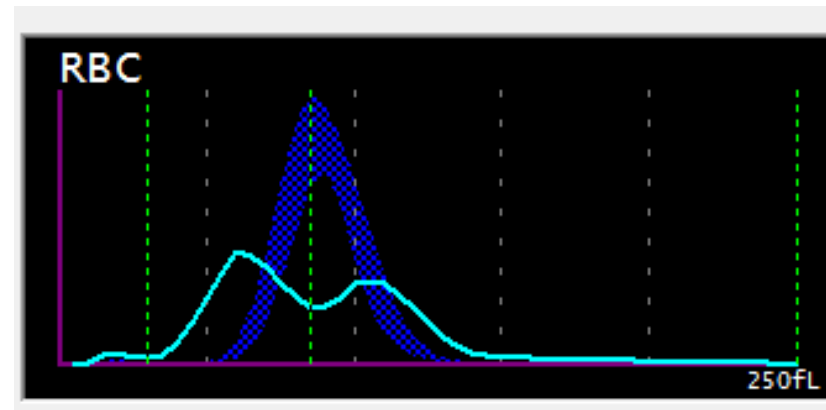
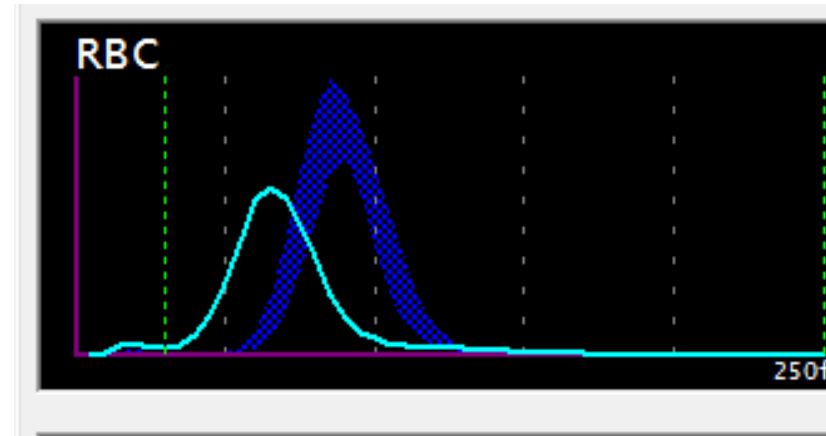
PLT Abn Dst

Next 303 Manual CBC+DIFF Xm



Cumulative data

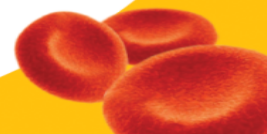
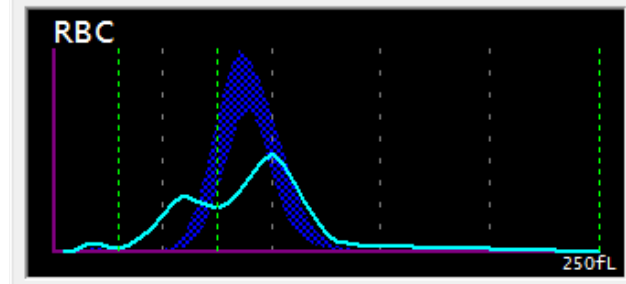
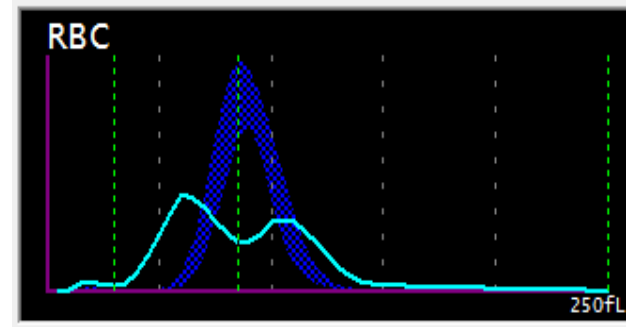
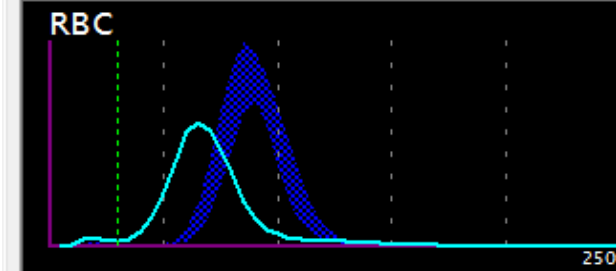
Cumulative Data			
Date	18/04	16/05	
Time	15:56	10:54	
No.	33	13	
WBC	8.39	7.04	
RBC	3.84	4.88	
HGB	8.4	11.0	
HCT	24.8	38.4	
MCV	64.6	78.7	
MCH	16.7	22.5	
MCHC	25.8	28.6	
PLT	462	365	
RDW-SD	43.3	----	
RDW-CV	19.3	32.0	
PDW	13.3	14.3	
MPV	10.7	10.8	
P-LCR	31.6	32.2	
PCT	0.49	0.40	



Follow up CBC Data

Cumulative Data

Date	18/04	16/05	06/06
Time	15:56	10:54	10:09
No.	33	13	20
WBC	8.39	7.04	7.37
RBC	3.84	4.88	4.78
HGB	8.4	11.0	11.9
HCT	24.8	38.4	39.3
MCV	64.6	78.7	82.2
MCH	16.7	22.5	24.9
MCHC	25.8	28.6	30.3
PLT	462	365	354
RDW-SD	43.3	----	----
RDW-CV	19.3	23.8	22.3
PDW	13.3	14.3	15.2
MPV	10.7	10.8	10.9
P-LCR	31.6	32.2	34.1
PCT	0.49	0.40	0.39



Case 2a – Health Check Up Sample 24/M

Data Browser 00-13 (Build 011) Logon Name: sysmex 2016/06/10(Fri) 18:28

Menu QC File Explorer Browser Switch Modify Validate Last 10 Output Upper Lower Delete

Positive 2016/06/10 18:18:48

Not Validated None

Main Graph Q-Flag Service Lab. Only

Item	Data	Unit	Item	Data	Unit	Item	Data	Unit
WBC	11.29 +	10 ³ /uL	NEUT#		10 ³ /uL	RET%		%
RBC	5.78 +	10 ⁶ /uL	LYMPH#		10 ³ /uL	RET#		10 ⁶ /uL
HGB	12.7	g/dL	MONO#		10 ³ /uL	IRF		%
HCT	37.7	%	EO#		10 ³ /uL	LFR		%
MCV	65.2 -	fL	BASO#		10 ³ /uL	MFR		%
MCH	22.0 -	pg	NEUT%		%	HFR		%
MCHC	33.7	g/dL	LYMPH%		%	RET-He		pg
PLT	210	10 ³ /uL	MONO%		%	IPF		%
RDW-SD	39.6	fL	EO%		%			
RDW-CV	18.1 +	%	BASO%		%			
PDW	13.7	fL	IG#		10 ³ /uL			
MPV	10.2	fL	IG%		%			
P-LCR	28.8	%						
PCT	0.21	%						

Flag(s)
Micro

Mode Manual

Data Browser 00-13 (Build 011) Logon Name: sysmex 2016/06/10(Fri) 18:37

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Positive 2016/06/10 18:18:48

Not Validated None

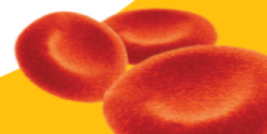
Main Graph Q-Flag Service Lab. Only

Action	Error/Rule Comments	Item	Data
	Review slide for low MCV	WBC	11.29 +
	Review slide for low MCH	RBC	5.78 +
	BTT Sehgal Index Positive	HGB	12.7
		HCT	37.7
		MCV	65.2 -
		MCH	22.0 -
		MCHC	33.7
		PLT	210
		RDW-CV	18.1 +

Flag(s)
Micro

Mode Manual

WB CBC DIFF RET >409



Sehgal Index for Beta Thalassemia

ORIGINAL ARTICLE

Sehgal index: A new index and its comparison with other complete blood count-based indices for screening of beta thalassemia trait in a tertiary care hospital

Kunal Sehgal, Preeti Mansukhani, Tina Dadu, Meher Irani, Shanaz Khodaiji

Department of Laboratory Medicine, P.D. Hinduja National Hospital and Medical Research Centre, Hematology Laboratory, Mumbai, Maharashtra, India

Address for correspondence:

Dr. Kunal Sehgal, Department of Laboratory Medicine, P.D. Hinduja National Hospital and Medical Research Centre, Hematology Laboratory, Mumbai, Maharashtra, India. E-mail: drkunalsehgal@gmail.com

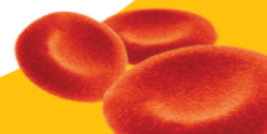
ABSTRACT

Introduction: Beta thalassemia trait (BTT) must be differentiated from iron deficiency anemia to avoid unnecessary iron therapy and for the prevention of thalassemia major by genetic counseling. In a tertiary care hospital, it is vital that the screening tool is not only sensitive but also specific so as to be cost effective and save time. **Aim:** The aim of this study was to evaluate the new Sehgal index and compare it to existing complete blood count-based indices for the best combination of sensitivity and specificity to predict BTT. **Materials and Methods:** Study was done in 2 phases - Phase 1: A retrospective analysis of 1022 consecutive high-performance liquid chromatography (HPLC) cases from July 2008 to June 2011. Phase 2: A prospective analysis of 973 consecutive HPLC cases from July 1, 2011 to June 10, 2013 was done to confirm the results of Phase 1 and the applicability of the new Sehgal index. **Results:** Prevalence of BTT was 28.8% (294/1022) and 25.39% (247/973) in Phase 1 and Phase 2, respectively. Receiver operating characteristic-area under the curve and Youden index was highest for new Sehgal index, followed by Mentzers index <14. The prospective study shows results similar to those in Phase 1 confirming the superiority of the above two indices. **Conclusion:**

Access this article online
Website: www.ijpmonline.org
DOI: 10.4103/0377-4929.162862
Quick Response Code:

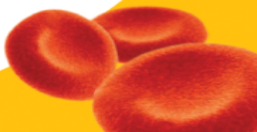

Routine testing to differentiate between IDA and BTT include: Complete blood count (CBC), serum iron, serum ferritin, total iron binding capacity (TIBC), bone marrow iron stores, levels of HbA2, free erythrocyte

Sehgal K et al Indian J Pathol Microbiol 2015;58:310-315



Screening for Beta Thalassemia Trait

- To evaluate and compare Sehgal index with the other CBC based indices for screening of BTT.
- Secondary objective: to use one of these formulas on a CBC analyser in a day to day practice to increase the pick up rate of BTT.
- More than 2000 CBC and HPLC Cases were evaluated

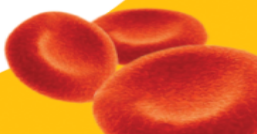


Sehgal Index for BTT

$$\text{Sehgal index} = \frac{\text{MCV} \times \text{MCV}}{\text{RBC}}$$

Sehgal Index < 972 – Suspect Beta Thalassemia Trait

Sehgal Index and Mentzers Index <14 had best combination of sensitivity and specificity for identifying BTT patients in a tertiary care hospital



XN L 350 RULES Screen

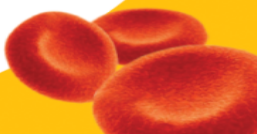
Rule [No. (Asc.):24] 00-13 (Build 011) Logon Name: sysmex 2016/06/10(Fri) 18:30

Menu QC File Explorer Browser Enable Display Output File

No.	Name	Action Comment	ItemVa
▶ 15	MCH >32	Review slide for high MCH	ItemVa
▶ 16	MCH <22	Review slide for low MCH	ItemVa
▶ 17	MCHC <30	Review slide for low MCHC	ItemVa
▶ 18	MCHC >36	Review slide for high MCHC	ItemVa
▶ 19	PLATELET <150	Review slide for low Platelet count	ItemVa
▶ 20	RBC >7000000	Review slide for High RBC	ItemVa
▶ 21	Platelet > 600000	Review slide for high Platelet	ItemVa
▶ 22	Beta Thal	BTT Sehgal Index Positive	ItemVa
▶ 23	Hb Hct mismatch	HCT > HB	ItemVa
▶ 24	HB HCT mismatch	HCT < HB	ItemVa

Comment Rule Validation Rule Output Rule

WB CBC DIFF RET >409 Mode Manual



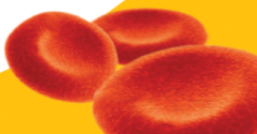
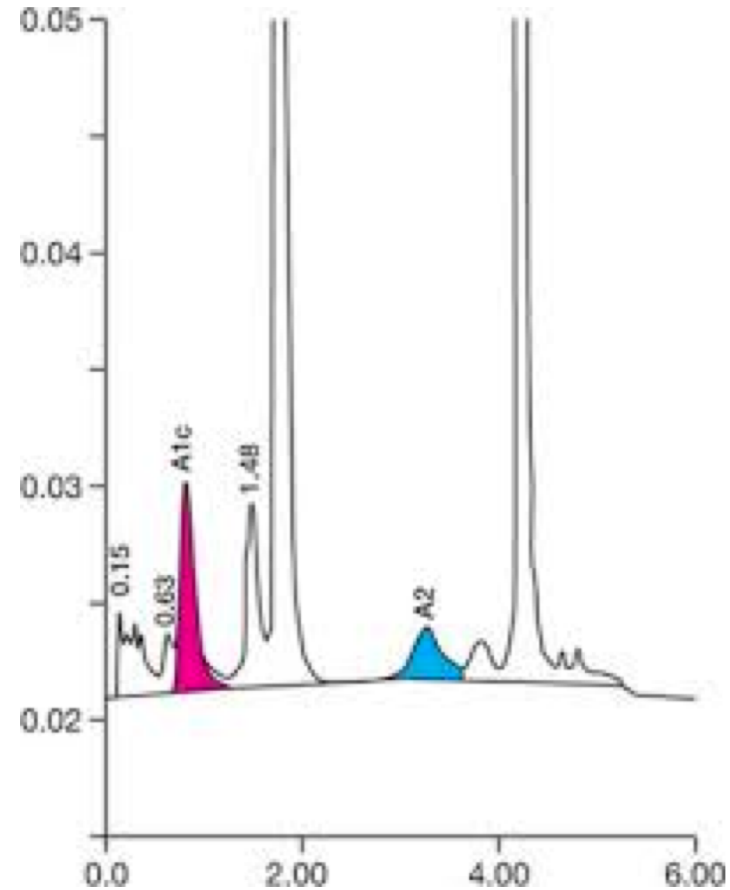
Suspected B Thal Trait – HbA2 levels by HPLC



Biorad D-10

Gold Standard for Hba1c Levels

Laboratory Standard for Hemoglobinopathies



Case 2b

Main | Graph | Cumulative | Q-Flags | Service | Research

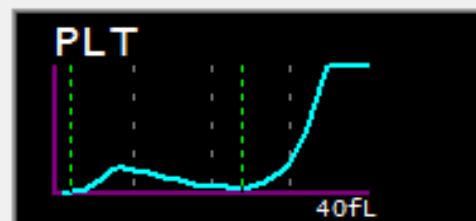
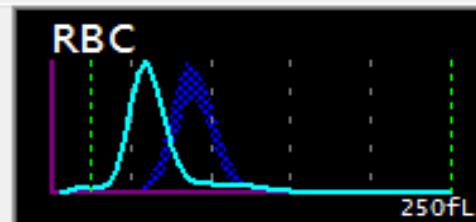
Items

Item	Data		Unit	LL	UL
WBC	11.95	+	10 ³ /uL		●
RBC	5.57	+	10 ⁶ /uL		●
HGB	9.9	-	g/dL	●	
HCT	31.7		%	●	
MCV	56.9	-	fL	●	
MCH	17.8	-	pg	●	
MCHC	31.2		g/dL	●	
PLT	341	*	10 ³ /uL		●
RDW-SD	36.0	-	fL	●	
RDW-CV	19.1	+	%		●
PDW	----		fL		
MPV	----		fL		
P-LCR	----		%		
PCT	----		%		

WBC Differential

Item	Data		Unit	LL	UL
NEUT#	8.64	+	10 ³ /uL		●
LYMPH#	2.25		10 ³ /uL	●	
MONO#	0.57		10 ³ /uL		●
EO#	0.46	+	10 ³ /uL		●
BASO#	0.03		10 ³ /uL	●	

Item	Data		Unit	LL	UL
NEUT%	72.3	+	%		●
LYMPH%	18.8	-	%	●	
MONO%	4.8		%	●	
EO%	3.8		%		●
BASO%	0.3		%	●	



Flag(s)

WBC

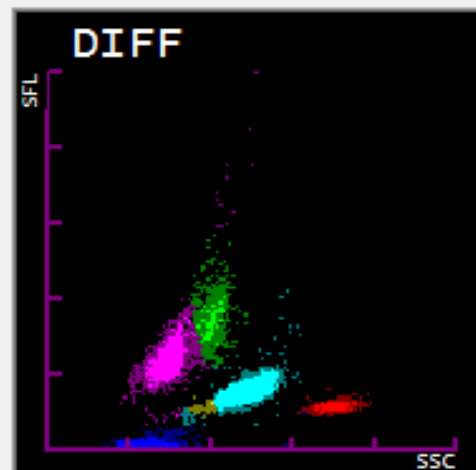
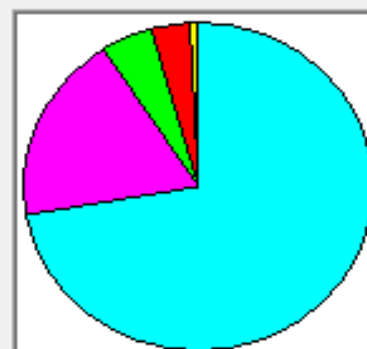
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RBC

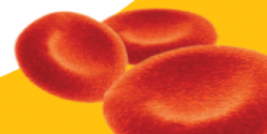
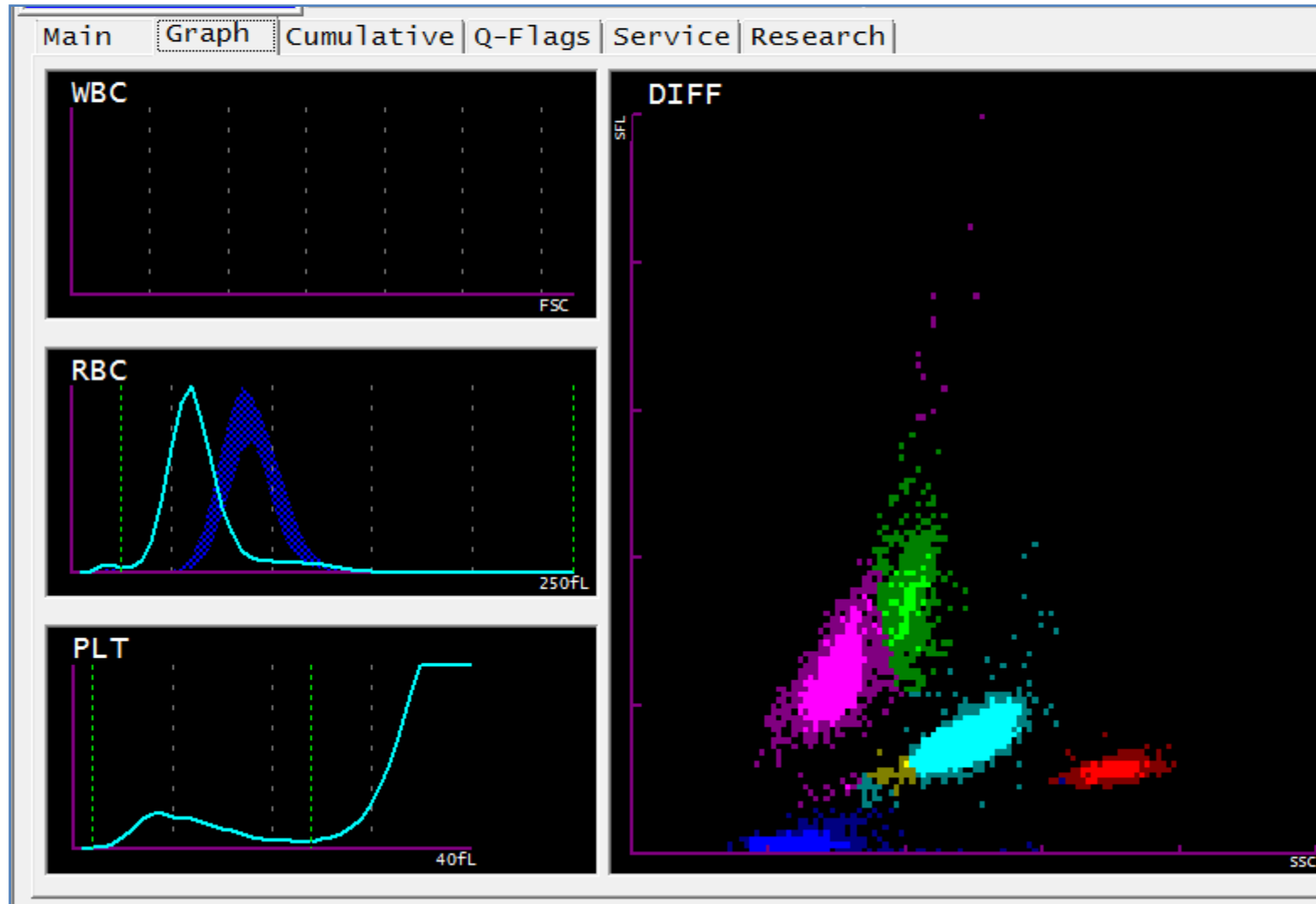
Aniso
Micro
Anemia

PLT

PLT Abn Dst



CONCOMITANT IRON DEFICIENCY & BTT



CASE 3

41 year old male

Employment Health Check Up

Tests Asked – CBC , Urine , Stool and HBsAg

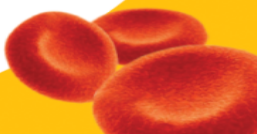
Clinically, the patient had raised BP at the time of presentation to OPD.

A routine CBC evaluation was done.

Hb 12.4gm/dl

WBC count 8490/cumm

Platelet count 1,26,000/cumm



CBC

Main | Graph | Cumulative | Q-Flags | Service | Research

Items

Item	Data	Unit	LL	UL
WBC	8.49	10 ³ /uL		
RBC	2.86 -	10 ⁶ /uL		
HGB	12.4	g/dL		
HCT	34.3 -	%		
MCV	119.9 +	fL		
MCH	43.4 +	pg		
MCHC	36.2 +	g/dL		
PLT	126 -	10 ³ /uL		
RDW-SD	53.1	fL		
RDW-CV	12.4	%		
PDW	10.0	fL		
MPV	10.2	fL		
P-LCR	27.1	%		
PCT	0.13 -	%		

WBC Differential

Item	Data	Unit	LL	UL
NEUT#	2.76	10 ³ /uL		
LYMPH#	4.17 +	10 ³ /uL		
MONO#	0.44	10 ³ /uL		
EO#	1.09 +	10 ³ /uL		
BASO#	0.03	10 ³ /uL		


Item	Data	Unit	LL	UL
NEUT%	32.5 -	%		
LYMPH%	49.1	%		
MONO%	5.2	%		
EO%	12.8 +	%		
BASO%	0.4	%		

Flag(s)

WBC: [Empty]

RBC: Macro

PLT: Thrombo-

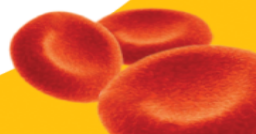


RBC Histogram (250fL)

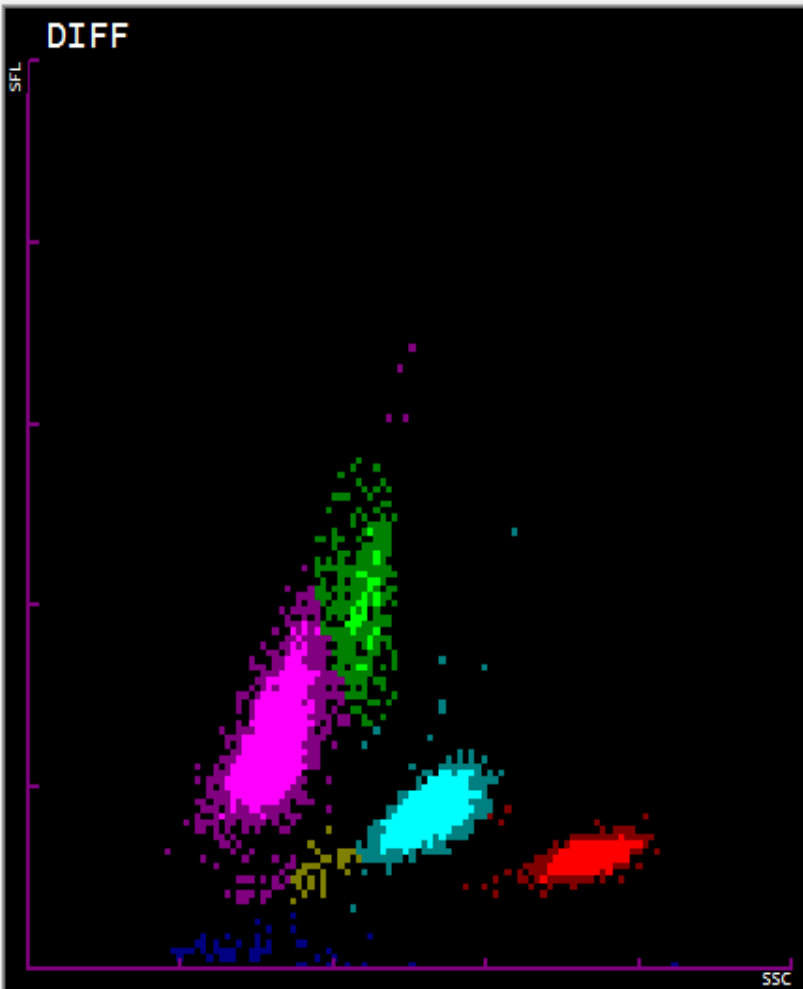
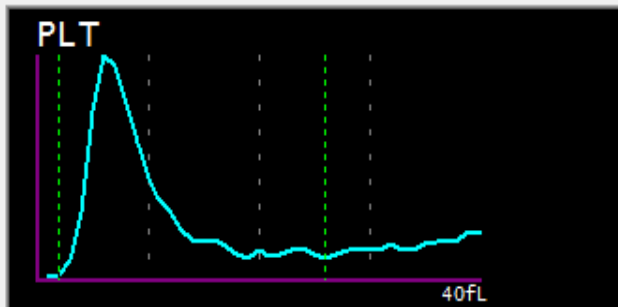
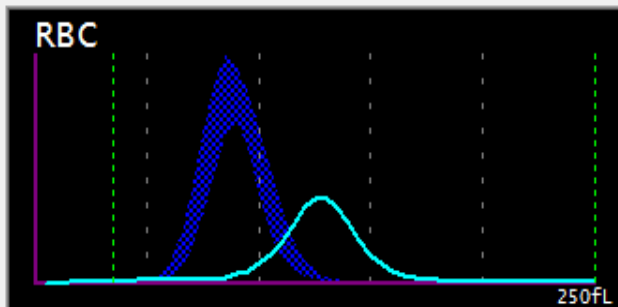
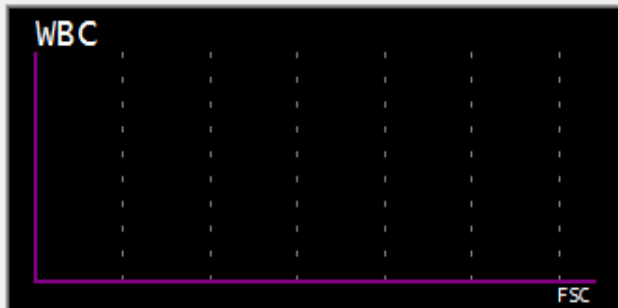
PLT Histogram (40fL)

DIFF Scatter Plot (SFL vs SSC)

XS Next 36 Manual CBC+DIFF Xm



Main Graph Cumulative Q-Flags Service Research



Flag(s)

WBC

RBC

Macro

PLT

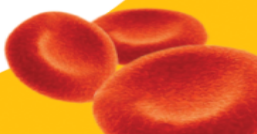
Thrombo-



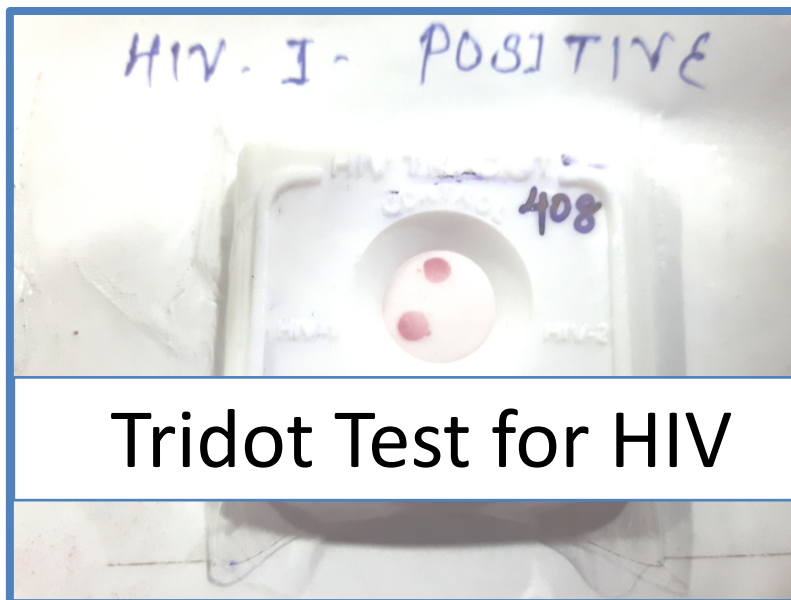
Peripheral smear findings



- RBC series : Predominantly Macrocytes mixed with few normocytic normochromic cells
- WBC series : Activated lymphocytes seen
- Platelets : Reduced on smear



MCV and Zidovudine Therapy



Tridot Test for HIV

Mean Corpuscular Volume as a Marker for Adherence to Zidovudine-Containing Therapy in HIV-Infected Adults

Joseph O. Mugisha¹, Katherine Donegan², Sarah Fidler³, Gita Ramjee⁴, Andrew Hodson³, David T. Dunn², Kholoud Porter², Pontiano Kaleebu¹ on behalf of the SPARTAC Trial Investigators[§]

¹MRC/UVRI Uganda Research Unit on AIDS, Entebbe, Uganda

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⁴MRC HIV Prevention Research Unit, Durban, South Africa

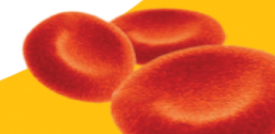
Abstract: *Objectives:* To assess whether mean corpuscular volume (MCV) is useful in detecting non-adherence to AZT-containing therapy.

Design: Observational study within randomised controlled trial.

Methods: We combined data from two treatment arms in SPARTAC, an RCT of short-course cART in primary HIV infection, classifying participants as responders (HIV-RNA decrease $\geq 1 \log_{10}$ or reaching <400 copies/ml) or non-responders following cART initiation. We assessed the sensitivity and specificity of using different percentage increases in MCV for accurately differentiating between responders and non-responders. We further examined changes in MCV levels up to 24 weeks after protocol-indicated cART cessation.

Results: Of 119 participants included in this analysis, 73 (61%) were women, 71 of whom were randomised in Africa. Ninety-eight (88%) and 84 (85%) were classified as responders at 4 and 12 weeks respectively following cART initiation. MCV increased by a mean 3% and 1% at week 4, and 14% and $<1\%$ at 12 weeks for responders and non-responders. A 2% MCV increase at 4 weeks had 62% sensitivity and specificity for identifying virological response. At 12 weeks, an 8% increase had 89% sensitivity and specificity. In responders, MCV remained lower for individuals in African compared to non-African sites throughout and rose from 85 vs 90 fL at cART start to 96 vs 103 fL at 12 weeks post-initiation then fell to 88 vs 93 fL and 86 vs 89 fL at 12 and 48 weeks post-cessation.

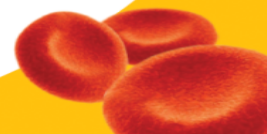
Conclusion: In low-income countries, where HIV RNA may be unavailable, 12-weekly MCV measurements may be useful in monitoring adherence to AZT-containing regimens.



CBC ADVANCE Report

<u>CBC ADVANCE</u>		
<u>HEMATOLOGY</u>		
<u>TEST</u>	<u>OBSERVED VALUE</u>	<u>REFERENCE RANGE</u>
Reticulocyte Count	3.30	0.42 - 1.82 %
RPI (Reticulocyte Production Index)	0.8	
Comments : RPI = Corrected reticulocyte count/ Reticulocyte maturation time in days RPI should be used only for adult anemic patients. RPI >2 indicates significantly increased hematopoiesis whereas RPI <2 indicates reduced response in an anemic patient.		
IRF (Immature Reticulocyte Fraction)	21.8	2.00 - 16.50 %
Comments : IRF gives an idea about the least mature erythrocytes which contain the most RNA. In many clinical situations, IRF increases before the total reticulocyte count and can be used to monitor BM response.		
Ret He (Reticulocyte Hemoglobin Equivalent)	23.8	28.7 - 34.1 pg
Comments : Ret He provides an indirect measure of functional iron over the last 3-4 days and is reduced in patients with functional iron deficiency. FID occurs when reticulo-endothelial stores are normal to high but iron is not delivered for erythropoiesis (eg. Chronic renal dialysis, Chronic inflammation, Cancer patients)		
IPF (Immature Platelet Fraction)	15.3	0.70 - 4.30 %
Comments : IPF (Platelet Reticulocyte count) is raised in patients with peripheral consumption/destruction of platelets (eg. ITP/TTP) and is normal or low in patients with BM failure. IPF can be used for predicting platelet count recovery post chemotherapy, stem cell transplant and dengue patients.		

*** END OF REPORT ***



XE 2100 - Normal Ranges - Publication

ORIGINAL ARTICLE

Reference range evaluation of complete blood count parameters with emphasis on newer research parameters on the complete blood count analyzer Sysmex XE-2100

Kunal K. Sehgal, Dadu Tina, Urmi Choksey¹, Reeta J. Dalal¹, Khodaiji J. Shanaz

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ABSTRACT

Since the advent of automation in the field of hematological cell counters there has been a constant refinement of the technology and increase in the number of newer parameters available on CBC analysers. Many novel parameters are being put into routine clinical use and both clinical evaluation and monitoring critically depend on knowledge of laboratory reference ranges. Here, we present reference interval for the Sysmex XE-2100, with emphasis on the novel or newer research parameters. Blood samples from a total of 122 clinically asymptomatic and apparently healthy subjects were evaluated and a final of 100 subjects (54-M, 46-F) were included in the study. A broad spectrum of parameters available with the analyser was assessed and reference ranges for the same evaluated.

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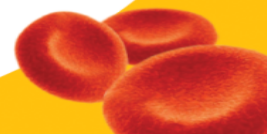
Website: www.ijpmonline.org

DOI: 10.4103/0377-4929.118698

Quick Response Code:

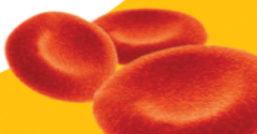
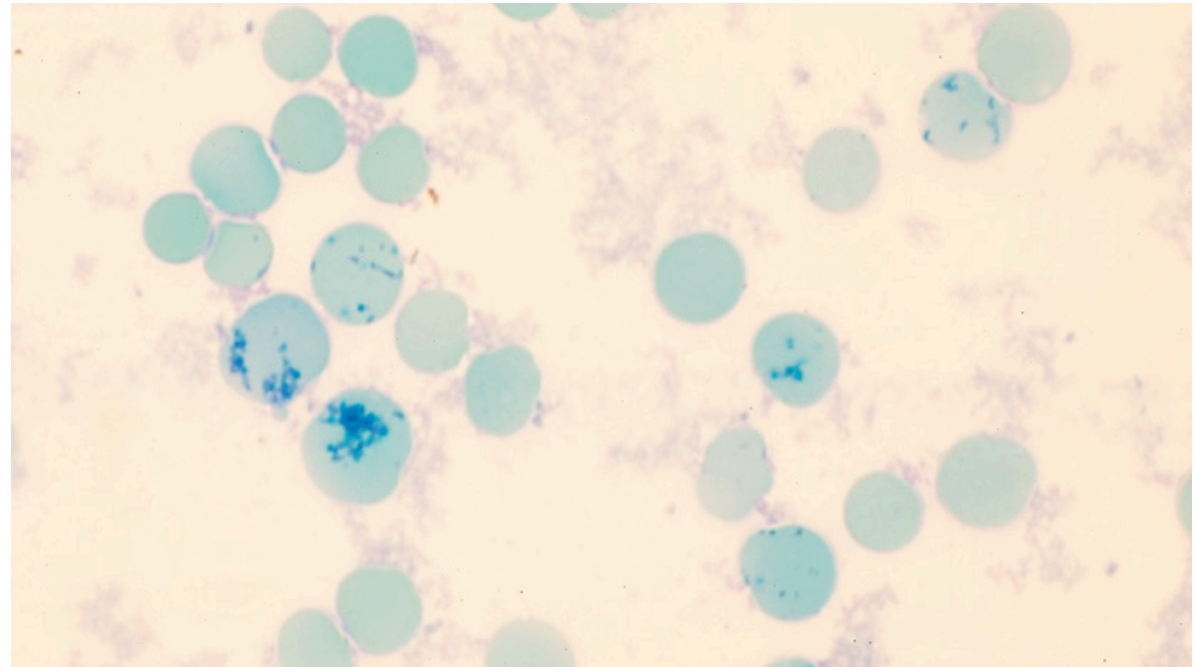


Sehgal K et al Indian J Pathol Microbiol 2013;56:120-4



Manual Reticulocyte Count

- Tedious
- Labour Intensive
- Subjective
- Very High CVs



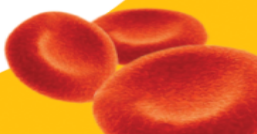
Automated Reticulocyte Count

PROS

- Rapid
- Reproducible
- Reliable
- Research parameters

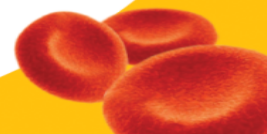
CONS

- Expensive
- Different machines use different dyes and techniques
- Standardisation is difficult
- Reference ranges to be established by every lab



Interpretation of Retic Count

High Retic Count	Low Retic Count
Blood Loss	Nutritional Deficiency- IDA, B12 deficiency
Hemolysis	Aplastic Anemia
Response to therapy	Post Chemo-radiation
Repopulating BM	BM infiltration- benign or malignant disorders



Normal Sample

Menu QC File Work List Explorer Browser Data Browser Modify Valid

Negative Rule Result 002 None 15/07/2015 10:28:29

Main Graph Cumulative Q-Flag Service User Lab. O

CBC

Item	Data	Unit	LL	UL
WBC	4.39	10 ³ /uL	●	
RBC	4.99	10 ⁶ /uL		●
HGB	13.5	g/dL		●
HCT	40.5	%		●
MCV	81.2	fL	●	
MCH	27.1	pg	●	
MCHC	33.3	g/dL		●
PLT &F	305	10 ³ /uL		●
RDW-SD	37.5	fL	●	
RDW-CV	12.9	%		●
PDW	10.9	fL	●	
MPV	9.9	fL	●	
P-LCR	23.8	%		●
PCT	0.28	%		●
NRBC#	0.00	10 ³ /uL		
NRBC%	0.0	%		

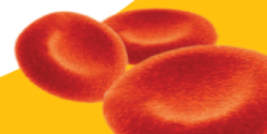
DIFF

Item	Data	Unit	LL	UL
NEUT#	2.26	10 ³ /uL	●	
LYMPH#	1.54	10 ³ /uL	●	
MONO#	0.35	10 ³ /uL		●
EO#	0.20	10 ³ /uL		●
BASO#	0.04	10 ³ /uL		●
NEUT%	51.4	%		●
LYMPH%	35.1	%		●
MONO%	8.0	%		●
EO%	4.6	%		●
BASO%	0.9	%		●
IG#	0.02	10 ³ /uL	●	
IG%	0.5	%	●	

RET

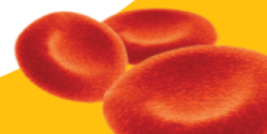
Item	Data	Unit	LL	UL
RET%	0.85	%	●	
RET#	0.0424	10 ⁶ /uL	●	
IRF	7.3	%	●	
LFR	92.7	%		●
MFR	6.8	%	●	
HFR	0.5	%	●	
RET-He	30.3	pg		●

Normal Ranges
 Reticulocyte % - 0.3%-1.5%, mean-0.9%
 IRF - 0.1-14.4%, mean-7.2%



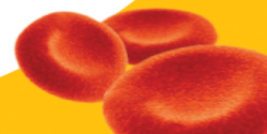
Case - Aplastic Anemia

Main	Graph	Cumulative	Q-Flag	Service
CBC				
Item	Data	Unit		
WBC	2.73 -	10 ³ /uL		
RBC	2.53	10 ⁶ /uL		
HGB	7.2 -	g/dL		
HCT	21.5 -	%		
MCV	85.0 -	fL		
MCH	28.5	pg		
MCHC	33.5	g/dL		
PLT &F	43 -	10 ³ /uL		
RDW-SD		fL		
RDW-CV	12.8	%		
PDW		fL		
MPV	11.1	fL		
P-LCR		%		
PCT		%		
NRBC#		10 ³ /uL		
NRBC%		%		
RET				
Item	Data	Unit		
RET%	0.23	%		
RET#		10 ⁶ /uL		
IRF	0.0	%		
LFR		%		
MFR		%		
HFR		%		
RET-He	34.1	pg		
DIFF				
Item	Data	Unit		
NEUT#	0.20 *	10 ³ /uL		
LYMPH#	2.41 *	10 ³ /uL		
MONO#	0.12 *	10 ³ /uL		
EO#	0.00	10 ³ /uL		
BASO#	0.00	10 ³ /uL		
NEUT%	7.3 *	%		
LYMPH%	88.3 *	%		
MONO%	4.4 *	%		
EO%	0.0	%		
BASO%	0.0	%		
IG#	0.01 *	10 ³ /uL		
IG%	0.4 *	%		
PLT-F				
Item	Data	Unit		
IPF	2.9	%		

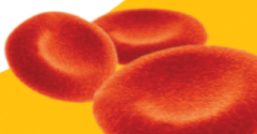
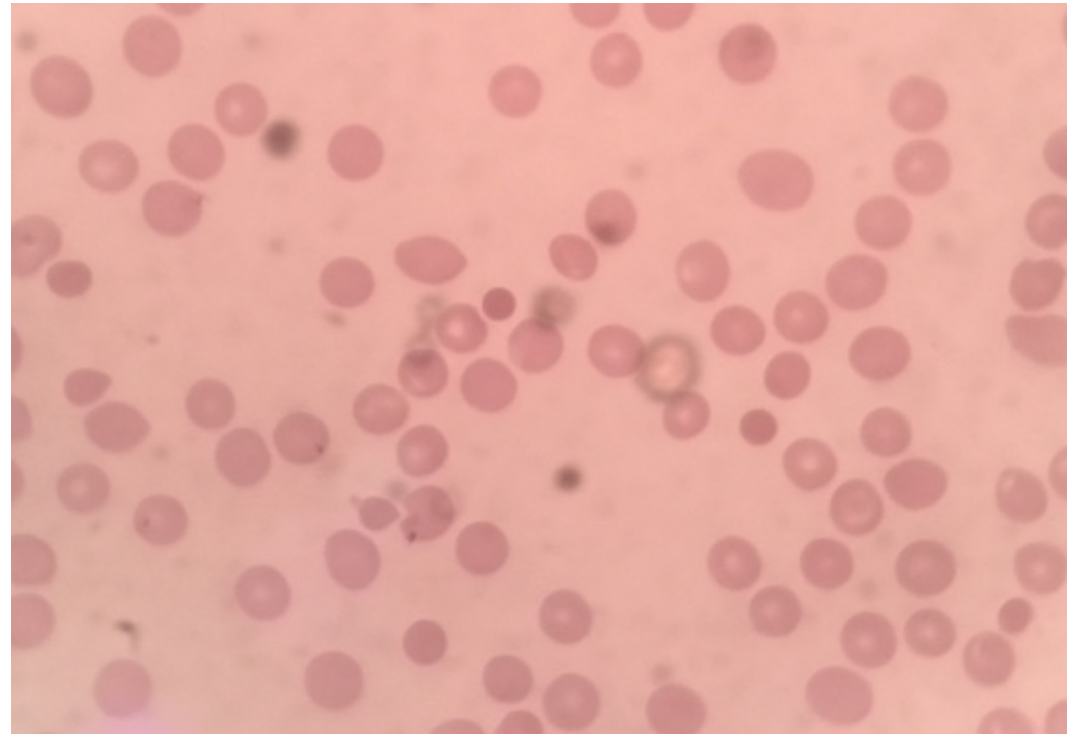
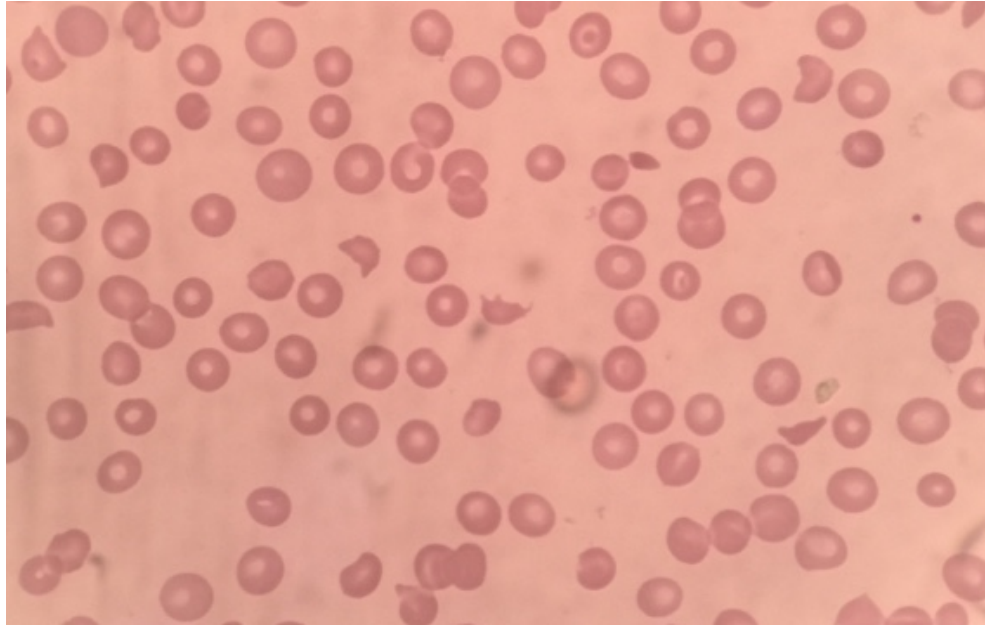


Case - 7/F Anemia - Hb-9, Retic-12.61%,

Cumulative	Q-Flag	Service	User	Lab. Only																																																												
Item	Data	Unit	<p style="text-align: center;">Normal Ranges</p> <p>Reticulocyte % - 0.3%-1.5%, mean-0.9%</p> <p>IRF - 0.1-14.4%, mean-7.2%</p> <p>RPI > 2</p> <p>Suggestive of blood Loss / Hemolysis</p> <p>RPI <2 – Inadequate Marrow Response</p>																																																													
WBC	11.91	10 ³ /uL																																																														
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MCH	31.6	pg																																																														
MCHC	33.7	g/dL																																																														
PLT-F	17	10 ³ /uL																																																														
RDW-SD	70.2	fL																																																														
RDW-CV	24.1 +	%																																																														
PDW	0.0	fL																																																														
MPV	----	fL																																																														
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RA-D%	0.2	%																																																														
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PLT-F	17	10 ³ /uL																																																														
H-IPF	5.0	%																																																														
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Microangiopathic Hemolytic Anemia



Automated Schistocyte Counts

International Journal of Laboratory Hematology

The Official Journal of the International Society for Laboratory Hematology



ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF LABORATORY HEMATOLOGY

ICSH recommendations for identification, diagnostic value, and quantitation of schistocytes

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^{‡‡}Daito Bunika University, Saitama, Japan

^{§§}Laboratory of Hematology, University Hospital and Groupe Français d'Hématologie Cellulaire (GFHC), Nancy, France

Correspondence:

Gina Zini, Research Center for Automated Methods in Hematology (ReCAMH), Service of Blood Transfusion, Institute of Hematology, Catholic University of Sacred Heart, Largo Agostino Gemelli, 8, 00168 Rome, Italy.

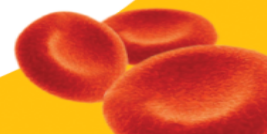
Tel.: +39 063015195;

Fax: +39 063055153;

E-mail: recamh@rm.uniscatt.it

SUMMARY

Schistocytes are fragments of red blood cells (RBCs) produced by extrinsic mechanical damage within the circulation. The detection of schistocytes is an important morphological clue to the diagnosis of thrombotic microangiopathic anemia (TMA). Reporting criteria between different laboratories, however, are not uniform, owing to variability of shape and nature of fragments, as well as subjectivity and heterogeneity in their morphological assessment. Lack of standardization may lead to inconsistency or misdiagnosis, thereby affecting treatment and clinical outcome. The Schistocyte Working Group of the International Council for Standardization in Haematology (ICSH) has prepared specific recommendations to standardize schistocyte identification, enumeration, and reporting. They deal with the type of smear, method of counting, morphological description based on positive criteria (helmet cells, small, irregular triangular, or crescent-shaped cells, pointed projections, and lack of central pallor). A schistocyte count has a definite clinical value for the diagnosis of TMA in the absence of additional severe red cell shape abnormalities, with a confident threshold value of 1%. Automated counting of RBC fragments is also recommended by the ICSH Working Group as a useful complement to the microscope, according to the high predictive value of negative results, but worthy of further research and with limits in quantitation.



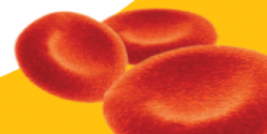
Case 6- 7/F Anemia - Hb-9, Retic-12.61%,

Cumulative	Q-Flag	Service	User	Lab. Only
Item	Data	Unit		
WBC	11.91	10 ³ /uL		
RBC	2.85	10 ⁶ /uL		
HGB	9.0	g/dL		
HCT	26.7	%		
MCV	93.7	fL		
MCH	31.6	pg		
MCHC	33.7	g/dL		
PLT-F	17	10 ³ /uL		
RDW-SD	70.2	fL		
RDW-CV	24.1 +	%		
PDW	0.0	fL		
MPV	----	fL		
P-LCR	0.0	%		
PCT	0.00	%		
NRBC%	0.8	%		
NRBC#	0.09	10 ³ /uL		
MicroR	8.2	%		
MacroR	9.5	%		
Delta-HGB	0.6 *	g/dL		
RBC-O	2.93	10 ⁶ /uL		
HGB-O	8.4 *	g/dL		
MCHC-O	31.5 *	g/dL		
FRC#	0.2029	10 ⁶ /uL		
RET%	12.61 *	%		
FRC%	7.12	%		
HYPO-He	5.9	%		
HYPER-He	0.2	%		

FRC % can be a valuable sensitive tool for follow up of patients of MHA to evaluate treatment response

Item	Data	Unit
RET%	12.61 *	%
RET#	0.3594	10 ⁶ /uL
IRF	21.1 *	%
LFR	78.9	%
MFR	13.1	%
HFR	8.0	%
RET-He	38.2 *	pg
RBC-He	27.2 *	pg
Delta-He	11.0 *	pg
RET-Y	187.8 *	ch
RET-RBC-Y	161.2 *	ch
IRF-Y	189.8 *	ch
FRC#	0.2029	10 ⁶ /uL
FRC%	7.12	%
HYPO-He	5.9	%
HYPER-He	0.2	%
RPI	3.9 *	
RET-UPP	0	
RET-TNC	180	

Item	Data	Unit
WBC	11.91	10 ³ /uL
IPF	12.9	%
PLT-I	17 *	10 ³ /uL
PLT-O	19	10 ³ /uL
PLT-F	17	10 ³ /uL
H-IPF	5.0	%
IPF#	2.2	10 ³ /uL



Normal ranges of Ret He

Ret He

- Range- 28.7 to 34.2 pg
- Mean 29.3pg

Literature

Ret He Normal Range- 28 to 35 pg

Thomas et al, Clinical Chem Lab Med 2005

Reference range evaluation of complete blood count parameters with emphasis on newer research parameters on the complete blood count analyzer Sysmex XE-2100

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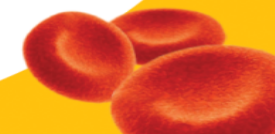
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ABSTRACT

Since the advent of automation in the field of hematological cell counters there has been a constant refinement of the technology and increase in the number of newer parameters available on CBC analysers. Many novel parameters are being put into routine clinical use and both clinical evaluation and monitoring critically depend on knowledge of laboratory reference ranges. Here, we present reference interval for the Sysmex XE-2100, with emphasis on the novel or newer research parameters. Blood samples from a total of 122 clinically asymptomatic and apparently healthy subjects were evaluated and a final of 100 subjects (54-M, 46-F) were included in the study. A broad spectrum of parameters available with the analyser was assessed and reference ranges for the same evaluated.

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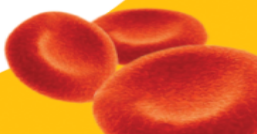
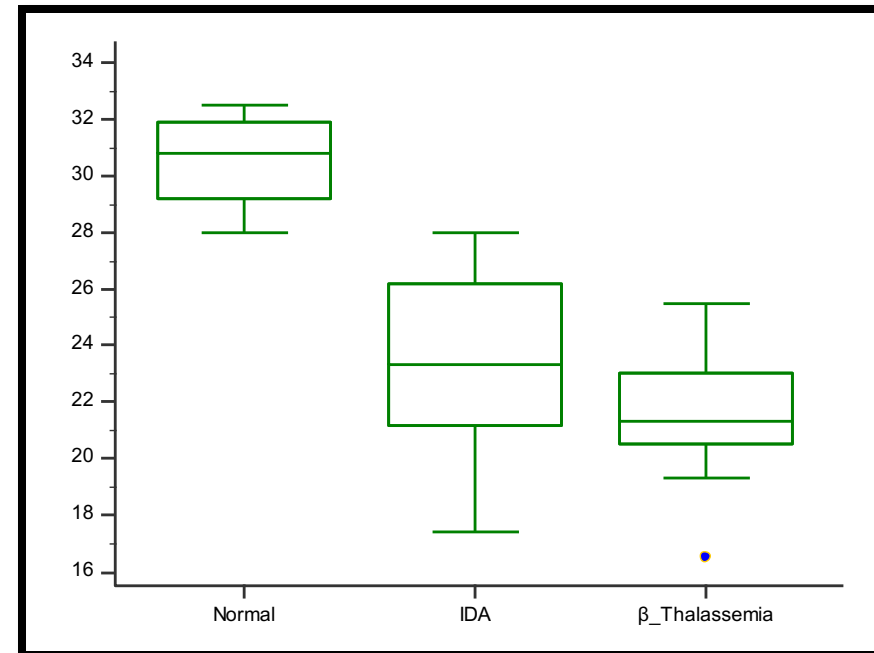


Ret He Evaluation – DNB Thesis Study

- To determine a cut off value of RET-He below which the patient can be said to have iron deficiency anemia for potential use in cases of Anemia of Chronic Disease

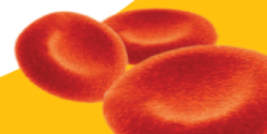
28.7-34.2 pg	18.3-28.7 pg	19.2-25.5 pg
Normal	IDA	BTT

- 184 Samples Evaluated
 - 96 normal samples
 - 71 IDA samples
 - 17 Beta Thalassemia samples



Ret He Cut offs for defining Iron Deficiency

Parameter for RET-He	Current study	Urrechaga et al	Brugnara et al	Canals et al
AUC	0.999	0.935	0.913	0.99
P value	<0.0001	<0.001	<0.0001	<0.001
Cutoff	28 pg	29.8 pg	27.2 pg	25 g



Case study - Ret He

NORMAL

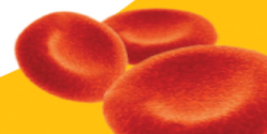
CBC		
Item	Data	Unit
WBC	4.39	10 ³ /uL
RBC	4.99	10 ⁶ /uL
HGB	13.5	g/dL
HCT	40.5	%
MCV	81.2	fL
MCH	27.1	pg
MCHC	33.3	g/dL
PLT &F	305	10 ³ /uL
RDW-SD	37.5	fL
RDW-CV	12.9	%
PDW	10.9	fL
MPV	9.9	fL
P-LCR	23.8	%
PCT	0.28	%
NRBC#	0.00	10 ³ /uL
NRBC%	0.0	%
RET		
Item	Data	Unit
RET%	0.85	%
RET#	0.0424	10 ⁶ /uL
IRF	7.3	%
LFR	92.7	%
MFR	6.8	%
HFR	0.5	%
RET-He	30.3	pg

Case of - IDA

CBC		
Item	Data	Unit
WBC	5.18	10 ³ /uL
RBC	3.89	10 ⁶ /uL
HGB	7.5	g/dL
HCT	25.9	%
MCV	66.6	fL
MCH	19.3	pg
MCHC	29.0	g/dL
PLT &F	183	10 ³ /uL
RDW-SD	53.4	fL
RDW-CV	24.1	%
PDW	----	fL
MPV	----	fL
P-LCR	----	%
PCT	----	%
NRBC#	0.01	10 ³ /uL
NRBC%	0.2	%
RET		
Item	Data	Unit
RET%	1.36	%
RET#	0.0529	10 ⁶ /uL
IRF	8.8	%
LFR	91.2	%
MFR	8.0	%
HFR	0.8	%
RET-He	24.9	pg

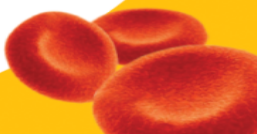
Case of- ACD

Item	Data	Unit
WBC	9.41	10 ³ /uL
RBC	3.09	10 ⁶ /uL
HGB	8.6	g/dL
HCT	27.5	%
MCV	89.0	fL
MCH	27.8	pg
MCHC	31.3	g/dL
PLT-F		10 ³ /uL
RDW-SD	47.3	fL
RDW-CV	15.0	%
PDW	8.3	fL
MPV	8.4	fL
P-LCR	11.2	%
PCT	0.24	%
NRBC%	0.0	%
NRBC#	0.00	10 ³ /uL
Item	Data	Unit
RET%	3.99	%
RET#	0.1233	10 ⁶ /uL
IRF	39.7	%
LFR	60.3	%
MFR	19.2	%
HFR	20.5	%
RET-He	25.8	pg

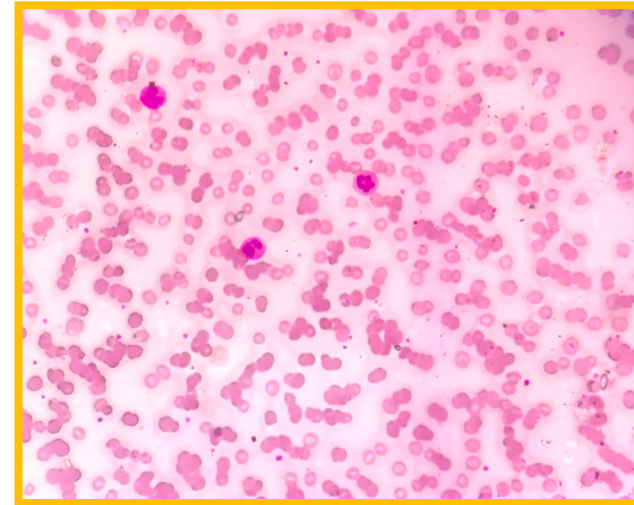


Case Study

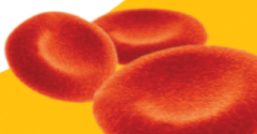
- 65/M
- Diabetic ,
- Anemia ,
- Body ache and vague Back pain
- High ESR



- CBC
 - Hb – 10.0g/dl
 - TLC – 7240 cells/ μ l
 - Platelet count – 1,68,000 / μ l
 - Peripheral smear shows Rouleaux formation
 - ESR 80mm at the end of one hour
- S. Creatinine – 1.3mg/dl

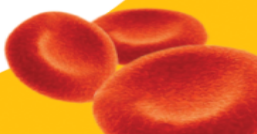


Rouleaux formation



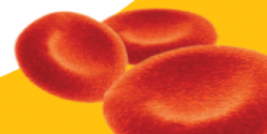
Initial workup

- Normal renal function tests
- Normal liver function tests
- Bence Jones Proteins, Urine Qualitative assay - **Negative**



S. Protein Electrophoresis

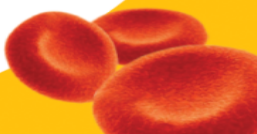
6 -- 7.8 gms/dl	Serum Protein	<u>10.2</u>
	Method : Biuret on fully automated system	
Result Rechecked		
4.02 -- 4.76 g/dl	Albumin (g/dl)	4.26
0.21 -- 0.35 g/dl	ALPHA 1 (g/dl)	0.32
0.51 -- 0.85 g/dl	ALPHA 2 (g/dl)	<u>1.03</u>
0.34 -- 0.52 g/dl	Beta 1 (g/dl)	0.52
0.23 -- 0.47 g/dl	Beta 2 (g/dl)	0.36
0.8 -- 1.35 g/dl	GAMA (g/dl)	<u>0.61 + 3.10 (M Band)</u>
	M Band	<u>M Band Observed In Gamma Region</u>
	Method : Capillary Electrophoresis.	
	Impression	<u>M Band Observed In Gamma Region</u>
	End of Report: Protein Electrophoresis	



S. Immunofixation qualitative

ALFA 1 REGION	Monoclonal Band not Detected
ALFA 2 REGION	Monoclonal Band not Detected
BETA 1 REGION	Monoclonal Band not Detected
BETA2 REGION	Monoclonal Band not Detected
GAMMA REGION	<u>Monoclonal band composed of</u>
M BAND	<u>IgG Kappa.</u> <u>Detected</u>

End of Report: IMMUNOFIXATION QUALITATIVE



S. Immunofixation quantitative

IMMUNOGLOBULIN G (mg/dl)

4497.0

Method : Immunoturbidimetric

IMMUNOGLOBULIN G (mg /dl) from Serum sample.

Total IMMUNOGLOBULIN A (mg/dl)

91.0

Method : Immunoturbidimetric

IMMUNOGLOBULIN A (mg /dl) from Serum sample.

BETA 2 MICROGLOBULIN (ng/ml)

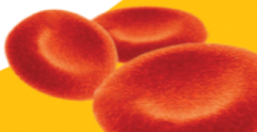
4477.0

Biological refernece interval :

Serum : 607 - 2164 ng/ml

Urine : Upto 300 ng/ml

Method : Fully automated CLIA.



S. Free Lite Chain Assay

Serum KAPPA Free Light chain (mg/L)	<u>594.7</u>
Result calculated by 1:10 Dilution	
Serum LAMBDA Free Light Chain (mg/L)	12.37
KAPPA/LAMBDA Free light chain ratio	<u>48.07</u>

Method : Immuno Turbidometry using reagents from Binding Site

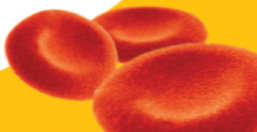
Reference Intervals:

Serum :

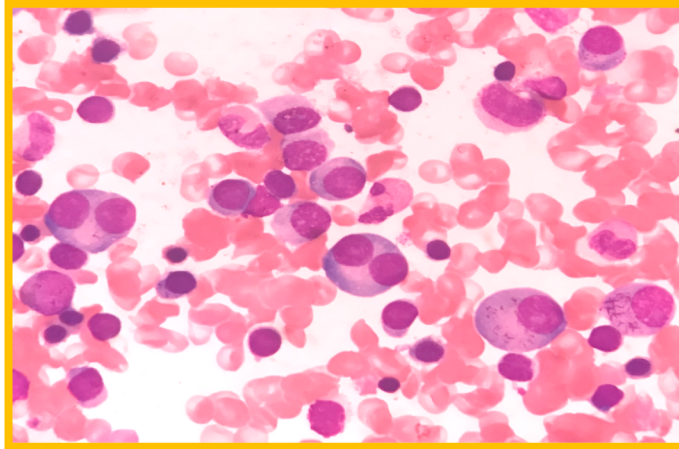
Free KAPPA : 3.3 - 19.4 mg/L

Free Lambda : 5.71 - 26.30 mg/L

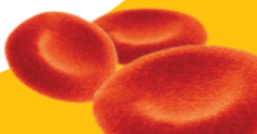
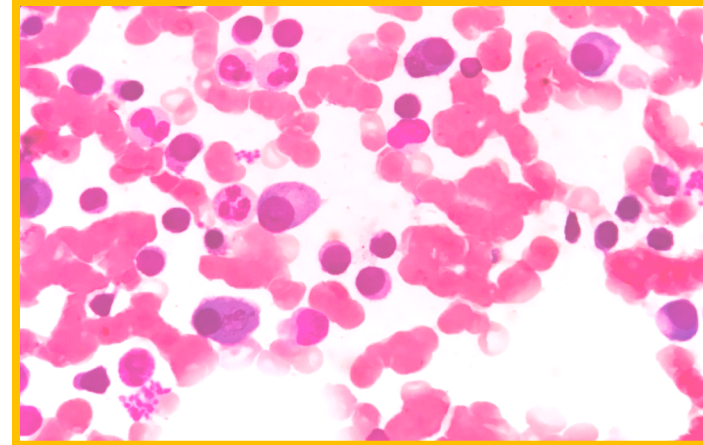
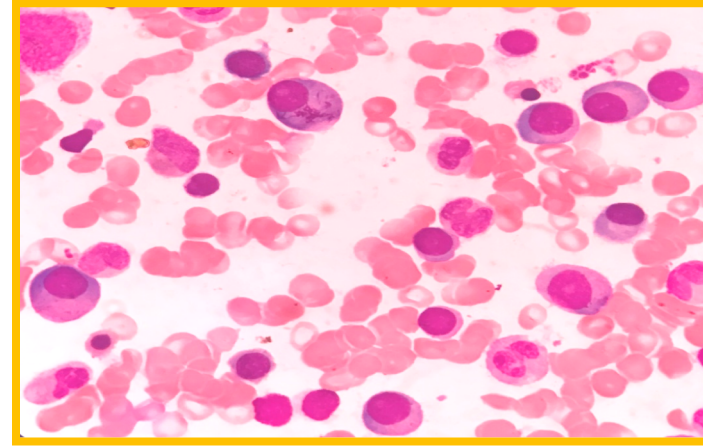
Free KAPPA/LAMBDA ratio : 0.26 - 1.65



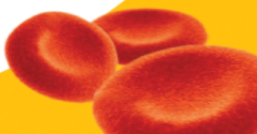
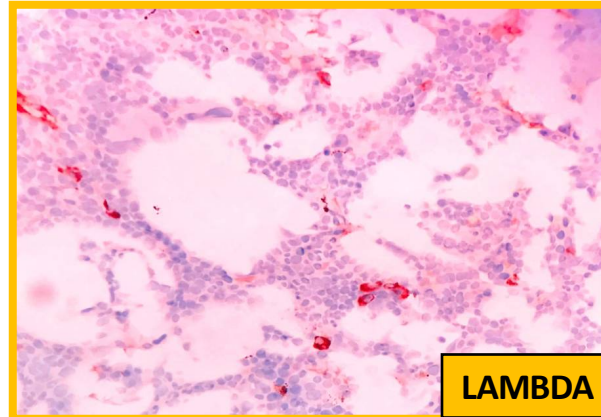
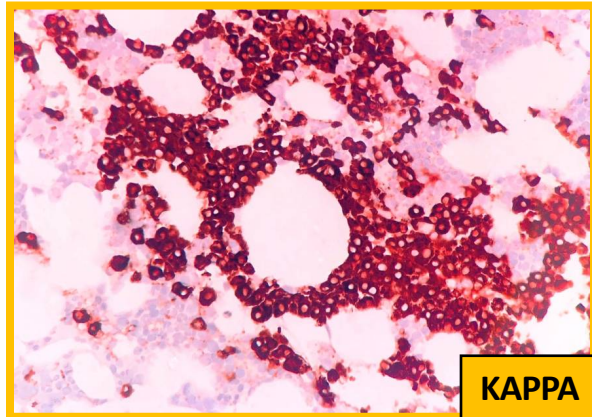
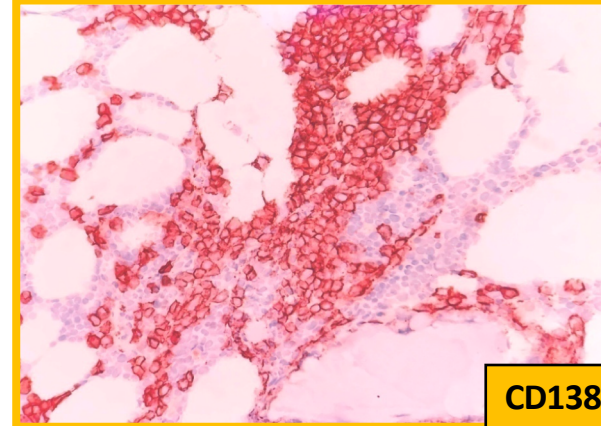
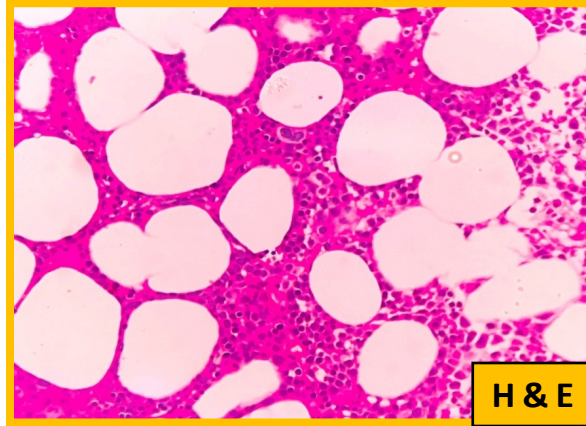
Bone marrow aspirate



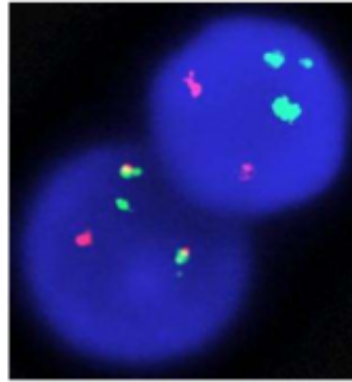
- Hypercellular marrow
- Reduced megakaryocytes
- Reduced erythroid series
- ~ 62% Plasma cells



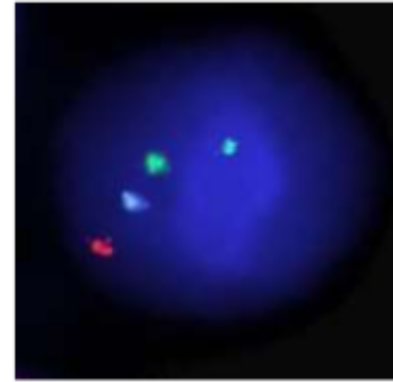
Bone marrow biopsy



iFISH analysis

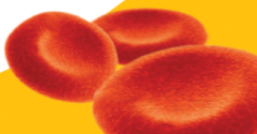


FISH on interphase cells
showing *IGH-FGFR3*
fusion: t(4;14)



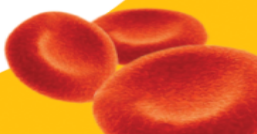
FISH on interphase cells
showing Monosomy of
chromosome 13

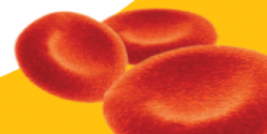
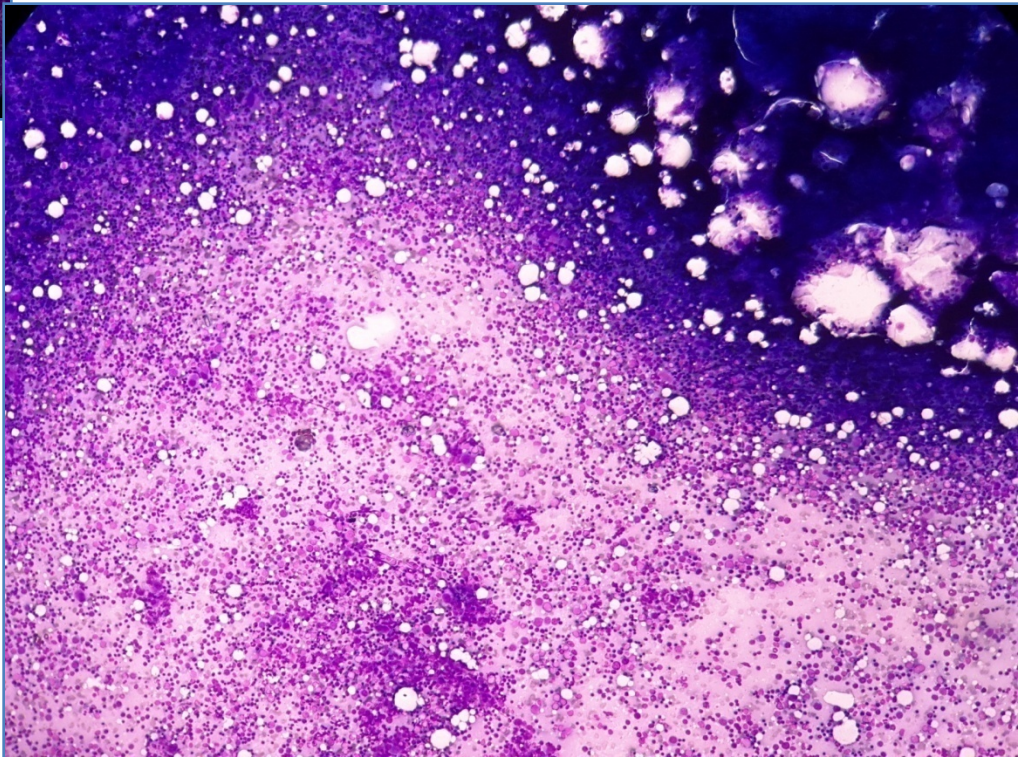
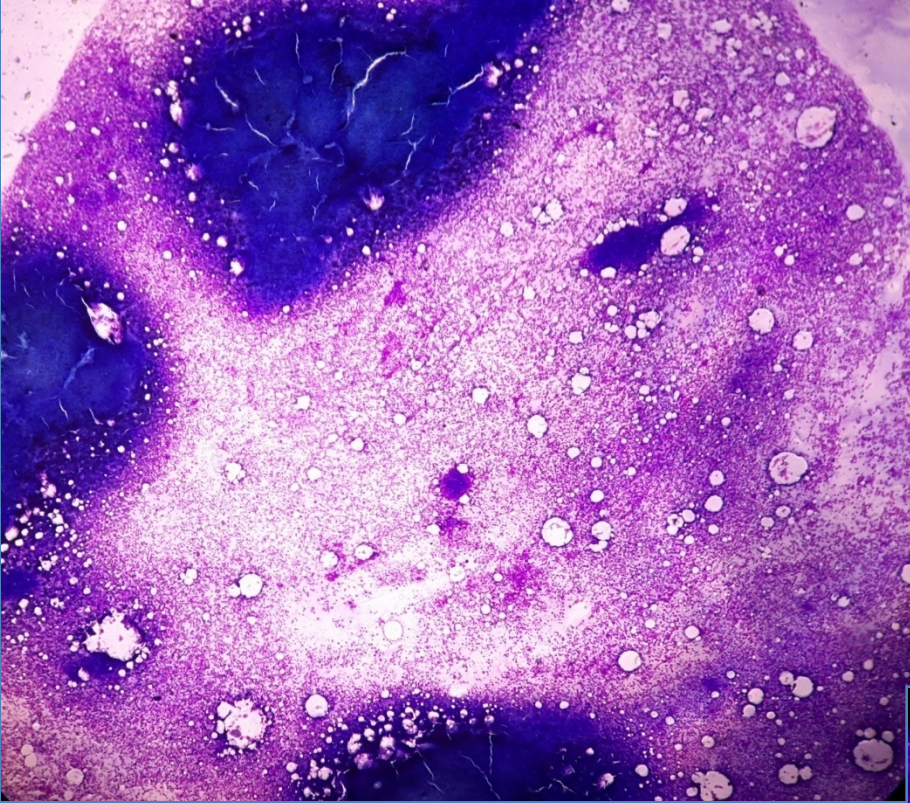
IMPRESSION: Hypodiploid MM with *IGH-FGFR3* Fusion: t(4;14)(p16;q32) and monosomy 13. The t(4;14) and monosomy 13 are high risk markers in MM. Hypodiploidy is associated with more aggressive disease in MM.

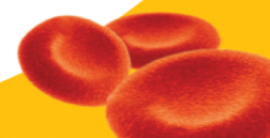
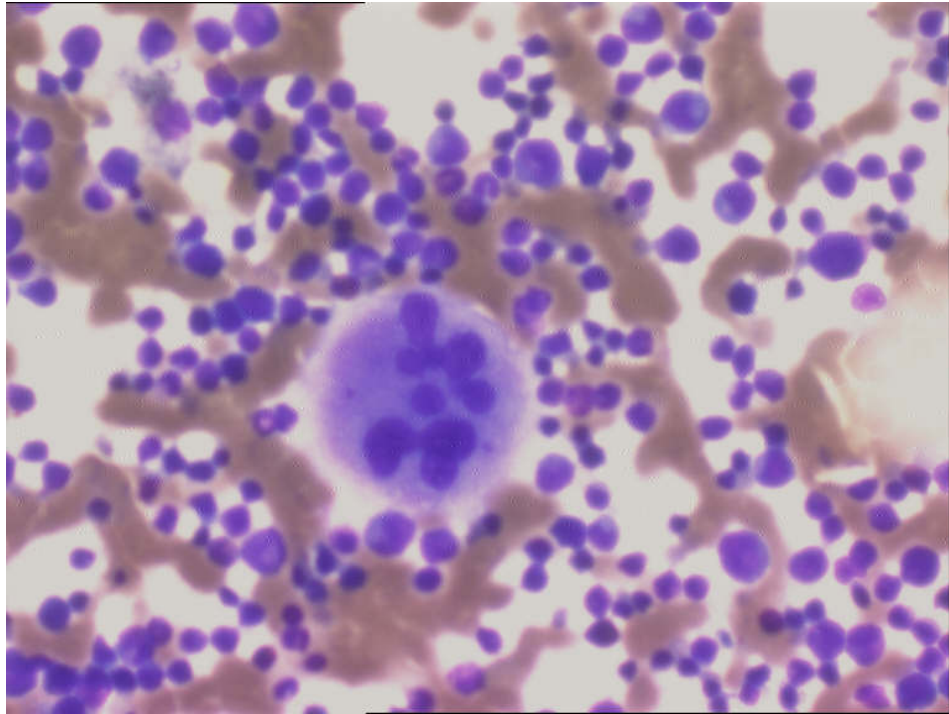
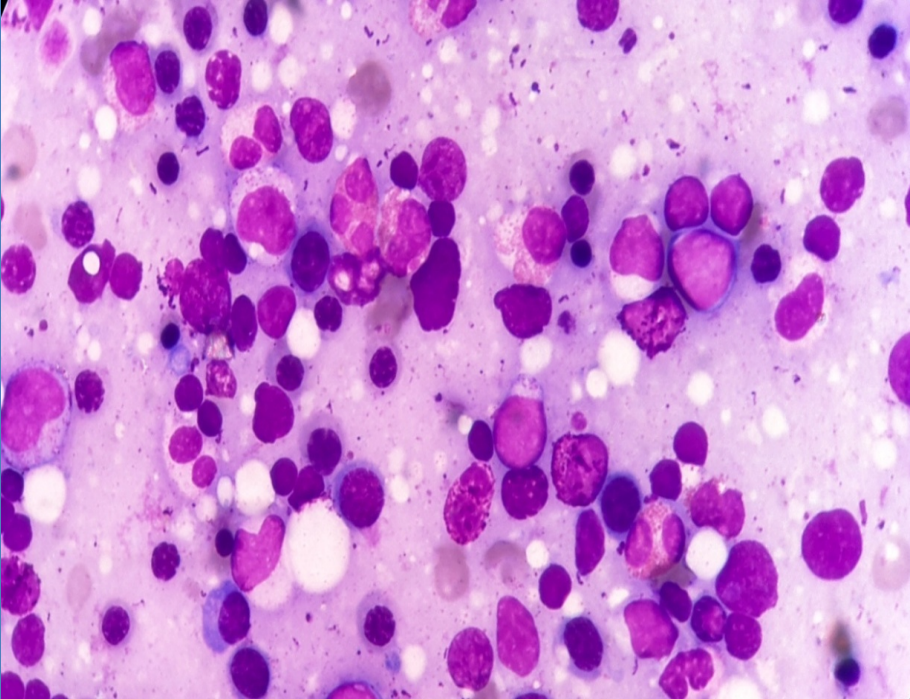
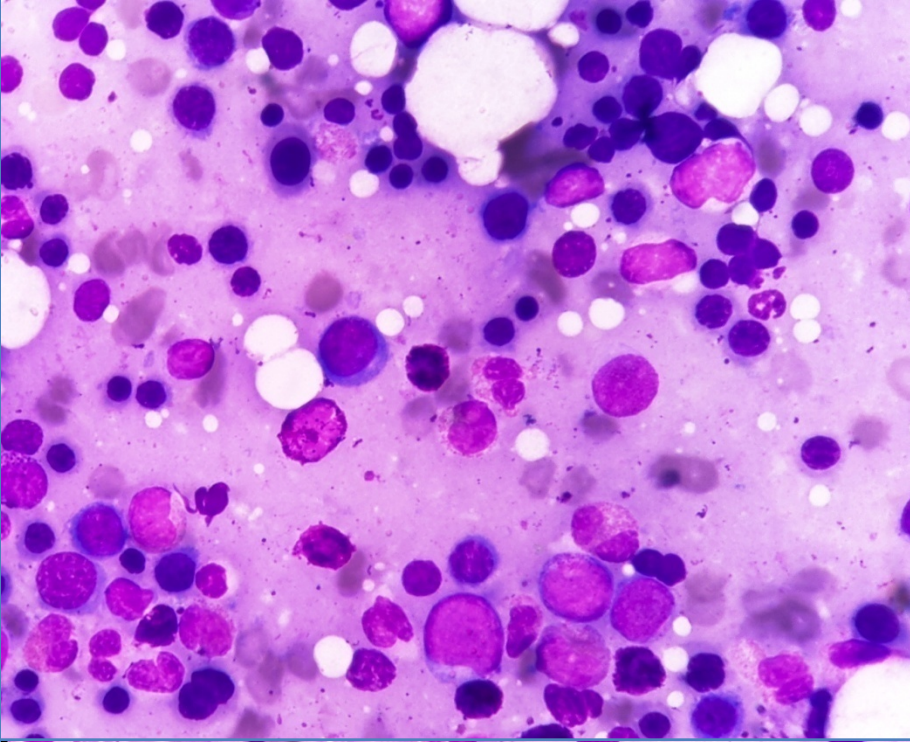


Case study

- 71 Year old male
- Pancytopenia
- Severe anemia
- Low . Vitamin B12 levels
- Not responding to hematinics
- Received multiple blood transfusions in recent past







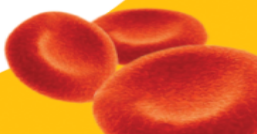
Bone Marrow Aspirate Report

Cellularity	- Partially hemodilute aspirate with markedly hypercellular imprint smears		
Megakaryocytes	- Adequate, Marked dysmegakaryopoiesis in form of hypolobation and lobe separation		
Erythroid Cells	- Increased, Mildly megaloblastic with mild dyserythropoiesis		
ME : E ratio	- 1:1.5		
Differential Count			
Blasts - 08	Promyelocytes - 02	Myelocytes - 03	Metamyelocytes – 02
Neutrophils – 05	Lymphocytes – 07	Monocytes – 01	Eosinophils – 07
Basophils – 09	Plasma cells – 01	Erythroid - 55	

IMPRESSION – Myeloid neoplasm with 8% blasts and prominent basophilia.

Differentials considered are – Myelodysplastic Syndrome with Excess of Blasts – 1 (MDS-EB1 – WHO 2016 classification) versus Evolving / Partially treated acute leukemia

Advise – Correlate with flow cytometry, cytogenetic and FISH reports



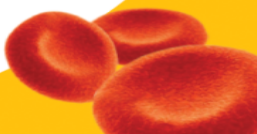
Flow Cytometry Evaluation

Descriptive summary - Flow cytometric immunophenotypic analyses of the bone marrow sample was done. A small population of blast ~8% is identified in the SSC/CD45 dot plot with medium SSC and dim to moderate CD45. These blasts express bright CD34 with dim CD13, CD33, HLADR and CD117. These blasts are negative for CD19, CD10, CD20 CD3, CD4, CD8, CD56 and CD64.

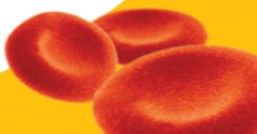
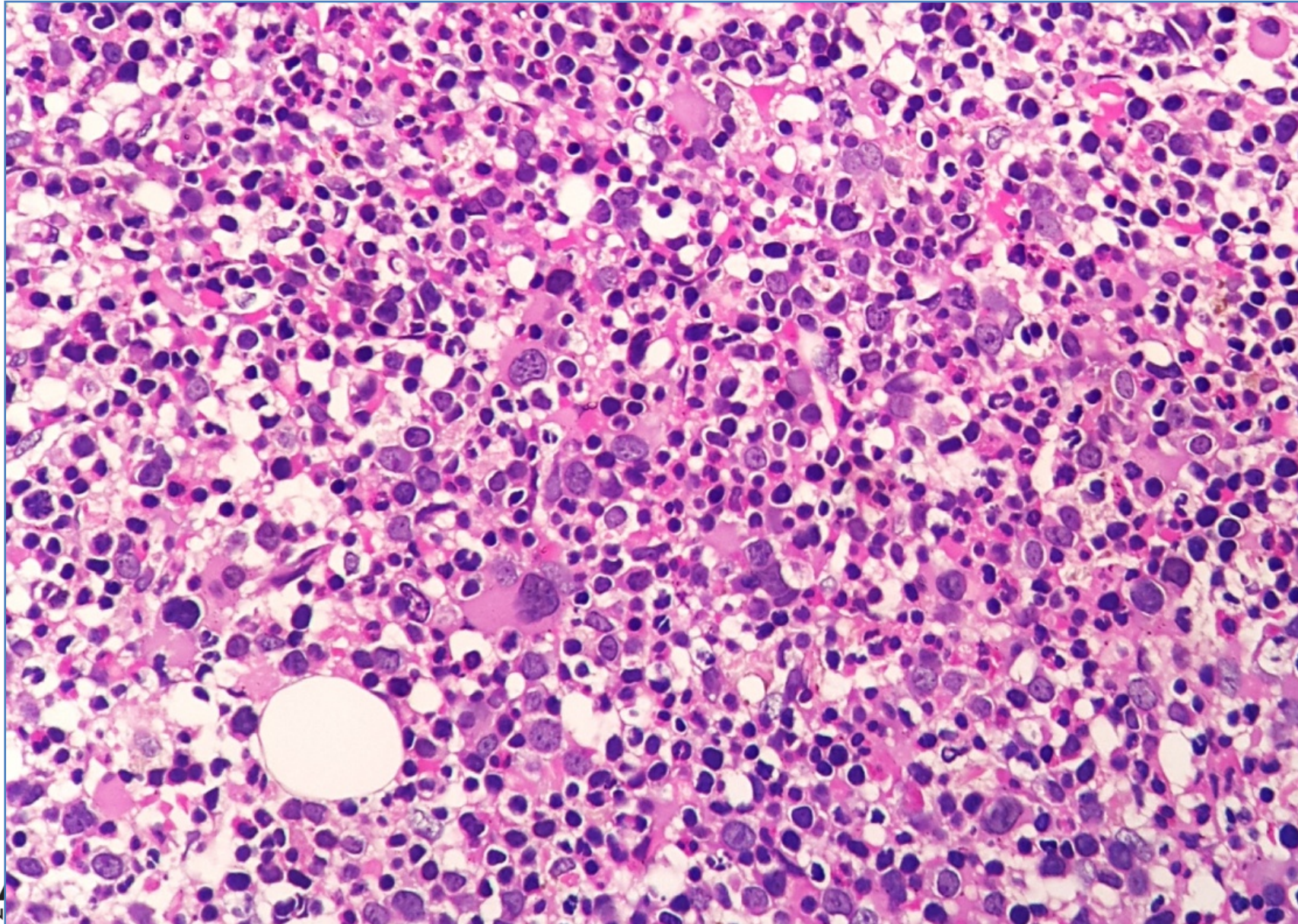
Population of basophils ~5% is also present along with few mast cells and scant population of monocytes. Population of lymphocytes is also seen.

Impression: Flow cytometry findings reveal 8% abnormal myeloid blasts along with 5% basophils indicative of a myeloid neoplasm.

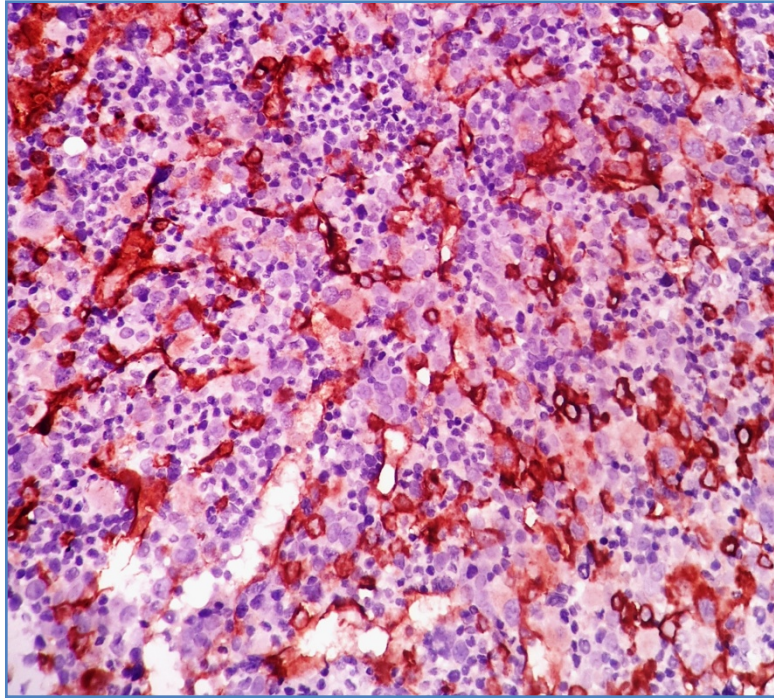
Overall bone marrow aspirate and immunophenotypic findings favour Myelodysplastic Syndrome with Excess of Blasts – 1 (MDS-EB1 – WHO 2016)



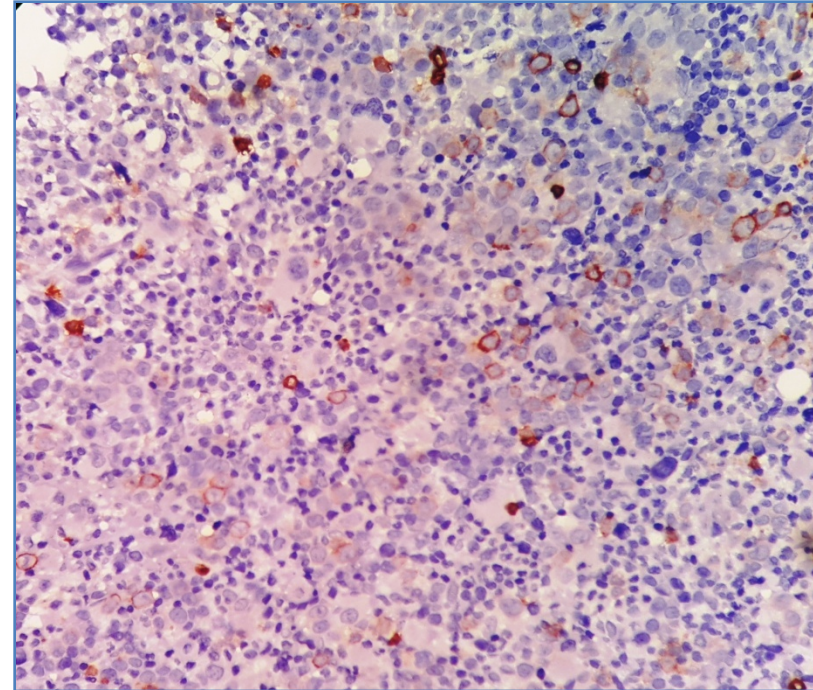
Bone Marrow Biopsy



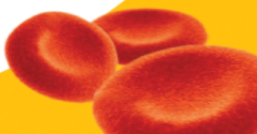
IHC



CD34



CD117



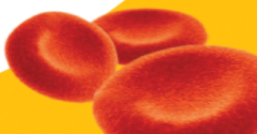
Bone Marrow Case Summary

- Clinical History – 71/M , severe pancytopenia, not responding to hematinics, requiring recurrent blood transfusions
- CBC- HB-7g/dl, WBC- 3.4×10^3 /ul, ANC- 1.2×10^3 /ul , Platelets- 70×10^3 /ul
- BMA- Hypercellular marrow with dysmegakaryopoiesis, megaloblastic dyserythropoiesis, dysmyelopoiesis with 8% blasts
- BM Biopsy- Hypercellular marrow with dysmegakaryopoiesis, megaloblastic erythropoiesis, CD34 & CD117 IHC showing increased blasts
- Flowcytometry- 8% abnormal myeloid blasts
- Karyotyping and FISH – Complex > 3 abnormalities

IMPRESSION – MDS–Excess of Blasts-1 (MDS-EB-1) (WHO 2016 classification)

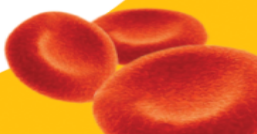
WPSS Score -5, Risk category- Very high, poor overall survival & increased risk for AML

IPSS-R Score -8, Risk category- Very high, poor overall survival & increased risk for AML



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- ANEMIA ON HEMATINIC THERAPY FOR RESPONSE
- ANEMIA OF CHRONIC DISEASE – CKD PATIENTS
- RECOVERY OF MARROW POST CHEMOTHERAPY OR VIRAL SUPPRESSION
- THROMBOCYTOPENIA
- PREDICTION OF PLATELET RECOVERY IN DENGUE
- SUSPECTED HEMATOLYMPHOID MALIGNANCIES
- SEVERE SEPSIS AND DIC



Thank You

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