Clinical utility of Reticulocyte Hemoglobin and Hypochromic erythrocytes reported by Mindray BC6800 Plus hematology analyzer in the study of erythropoiesis

ifcc International Federation of Clinical Chemistry and Laboratory Medicine

EFLM EUROPEAN FEDERATION OF CLINICAL CHEMISTRY AND LABORATORY MEDICINE

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6000 **27** Research Parameters，6800Plus **42** Research Parameters

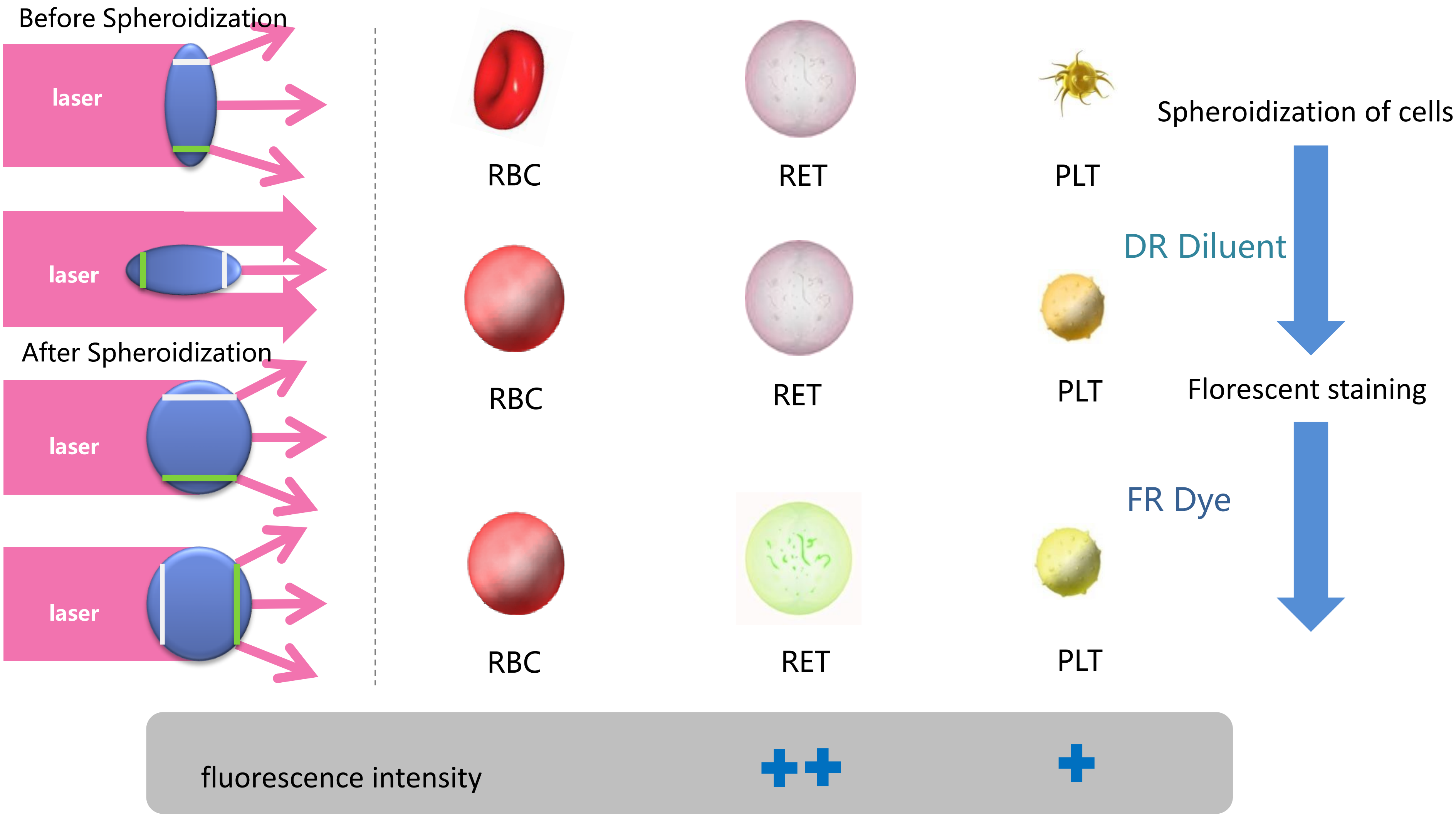
6000
27

→

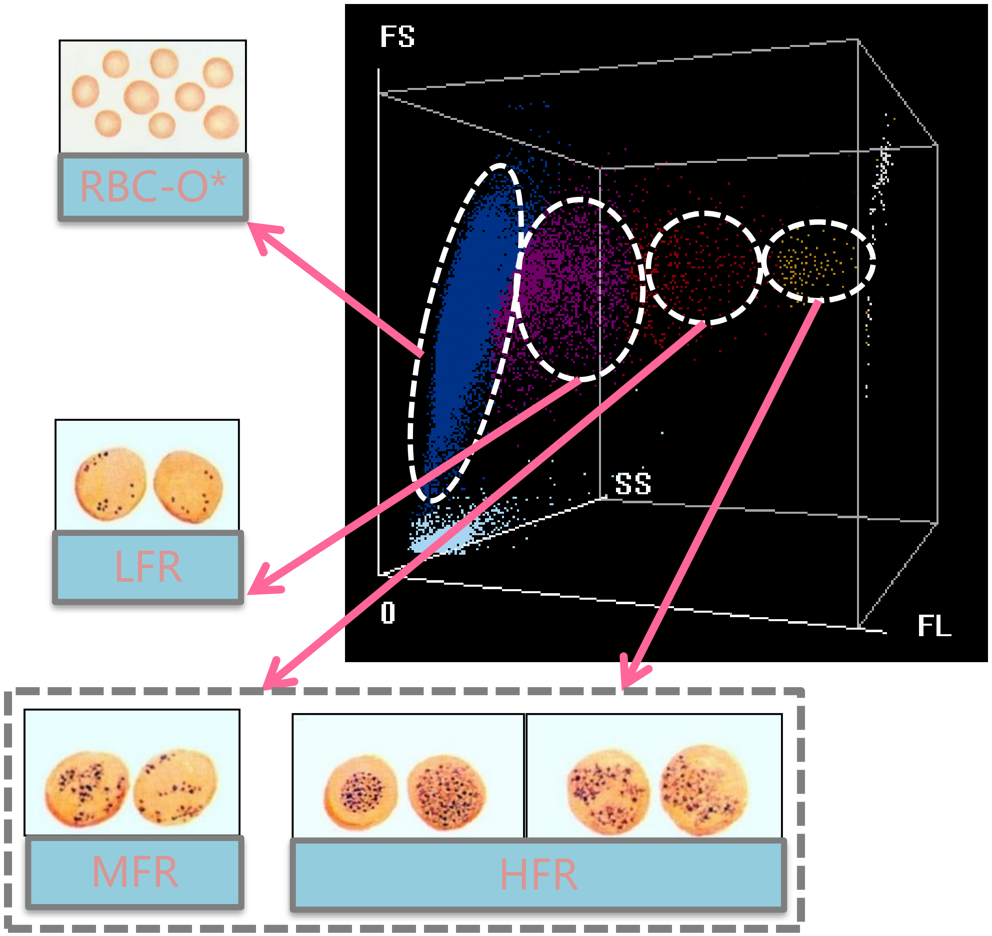
6800PLUS
42

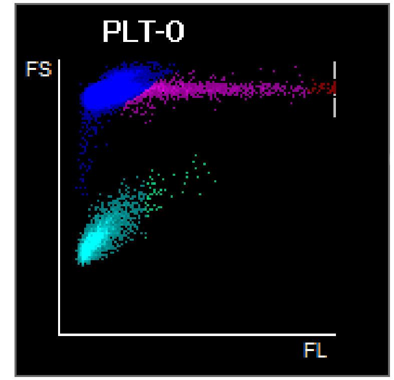
WBC-O					Neu-X	Lym-X	Mon-X	PLT-O	
WBC-D	TNC-D	HFC#	IME#	NLR	Neu-Y	Lym-Y	Mon-Y	PLT-I	H-IPF
WBC-N	TNC-N	HFC%	IME%	PLR	Neu-Z	Lym-Z	Mon-Z	PDW-SD	IPF#
RBC-O	H-NR%	Micro#	Macro#	INR#	SRBC	SMCV	FRC#	MRV	RPI
	L-NR%	Micro%	Macro %	INR%	LRBC	LMCV	FRC%	Hypo%	RHE CHr

RET Channel —— Cell Processing Method



RET Channel—Effectively Avoid The Interference of Abnormal Samples to RBC/PLT Results





IPF (Immature Platelet Fraction)

can guide Platelet transfusion after stem cell transplantation, and may also be used as an index of thrombopoietic activity in bone marrow.

HFR: High Flourescence Reticulocytes

RHE: (reticulocyte hemoglobin content) Provides clinical information for differential diagnosis and monitoring of erythopoiesis

RBCs 3D 9-square Graph

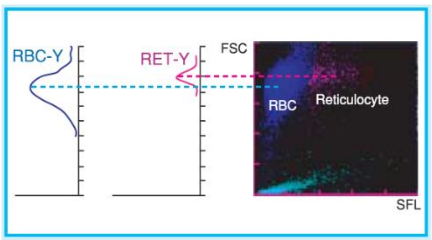
Demands：

1、 Using Mie scatter to calculate the single Corpuscular Hemoglobin (RBCs 3D 9-square Graph) by Siemens now is recognized by the industry.

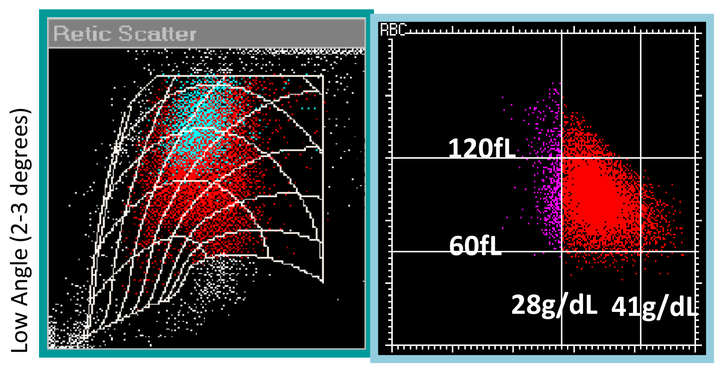
2、 Reticulocyte hemoglobin has specific value on clinical treatment of iron deficiency anemia, and differential diagnosis of iron deficiency anemia / thalassemia.

Present situation：

Sysmex XN： FSC calculates HGB



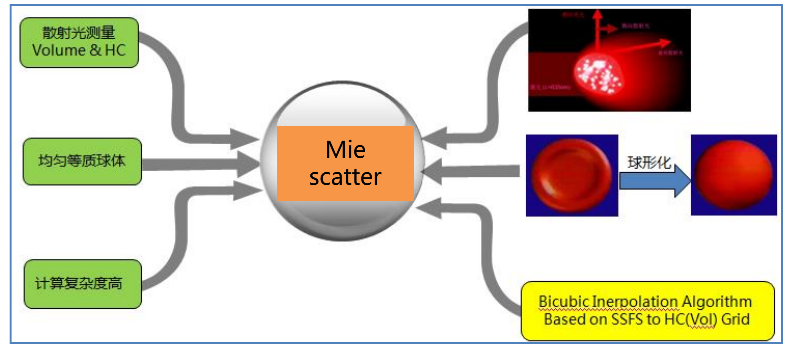
ADVIA 2120i： Mie scatter calculates RBC volume /HGB

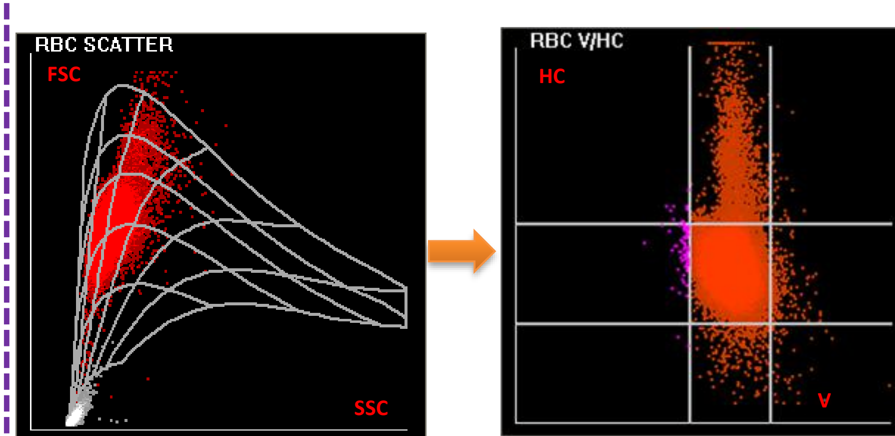


High Angle (5-15 degrees) HC

Advance hematology analyzer：

Mie scatter calculates Single Corpuscular Hemoglobin Concentration/volume

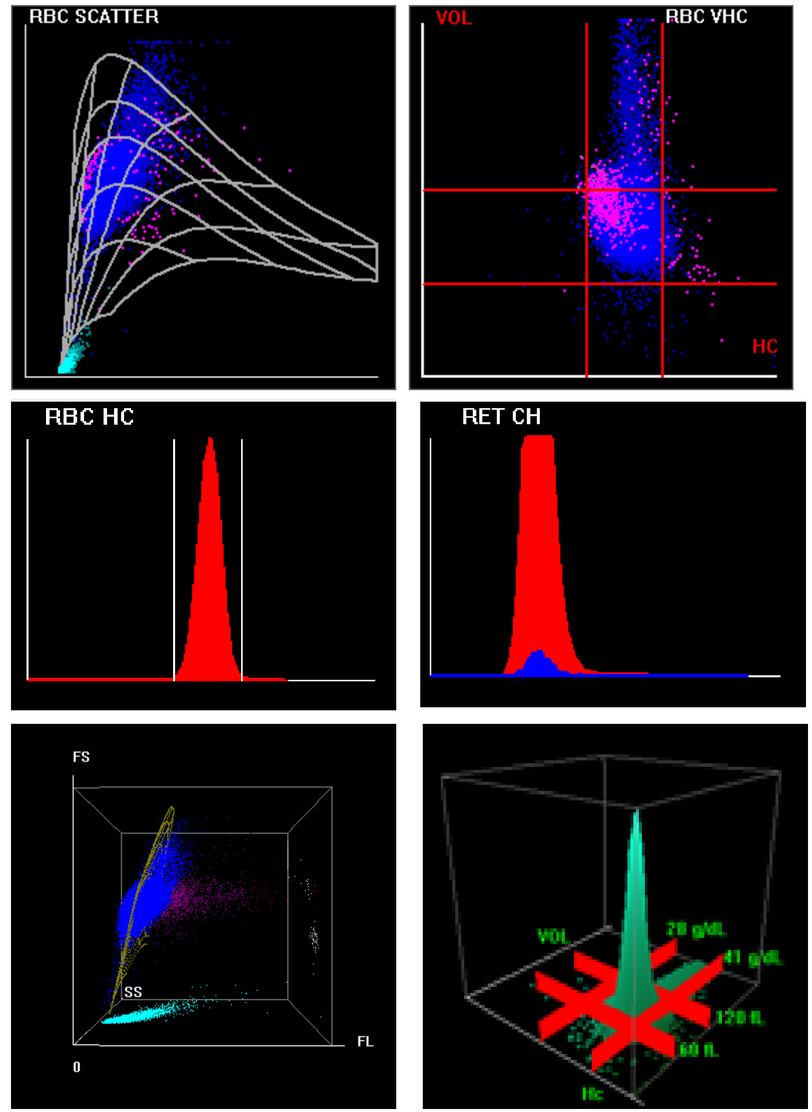




raw scatter gram 9-square graph

Meaning	Advanced Hematology analyzer
Mean Corpuscular Hemoglobin Concentration	MCHC
Single Corpuscular Hemoglobin Concentration	MCH-O(RUO)
HGB Distribution Width	HDW
Reticulocyte Hemoglobin Expression	RHE (MCHr,RH,RCH)
Mean Reticulocyte Volume	MVCr （RCV,RMCV）
Mean Platelet Component Conc,	MPC
Microcyte count	Micro#
Microcyte percentage	Micro%
Macrocyte count	Macro#
Macrocyte percentage	Macro%
The percentage of hyperchromic red blood cells	HYPER%
The percentage of hypochromic red blood cells	HYPO%
Reticulocyte Production Index	RPI

RBCs 3D 9-square Graph



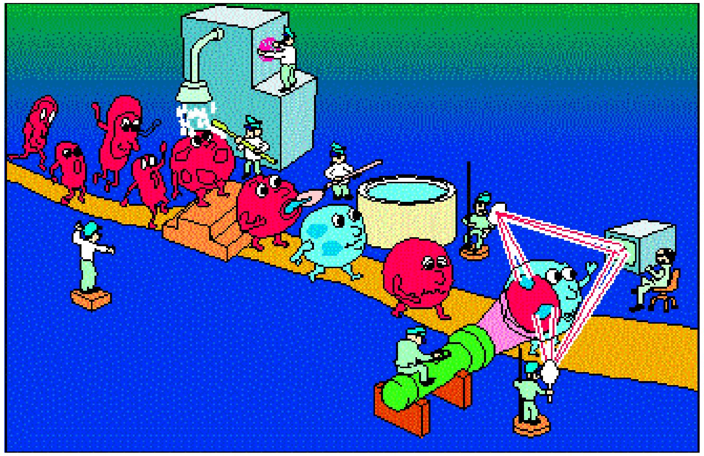
Innovative erythrocyte parameters

These innovative parameters most typically include automated reticulocyte and nucleated RBC counts,

hemoglobinization of reticulocytes and RBC

reticulocyte maturation

automatic analysis and **calculation of microcytic and hypochromic RBC**



The various combination of these different parameters not only may be useful to complement clinical history, physical examination and results of more conventional laboratory investigations for investigating the underlying cause(s) of anemia.

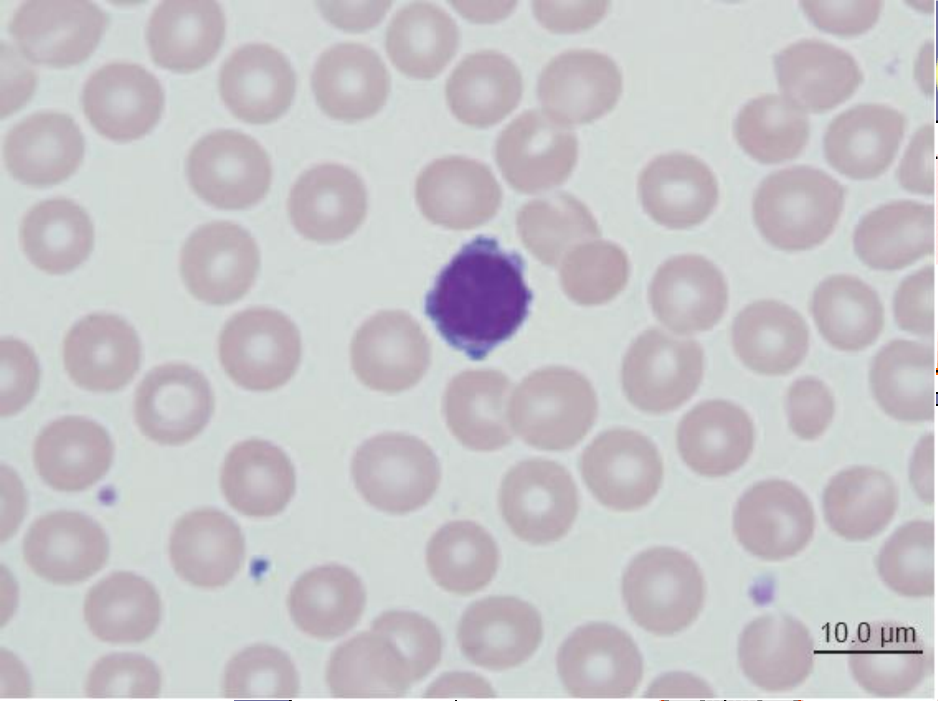
G Lippi & M Plebani. Recent developments and innovations in red blood cells diagnostics
Journal of Laboratory and Precision Medicine 2018DOI: 10.21037/jlpm.2018.07.09 AOP

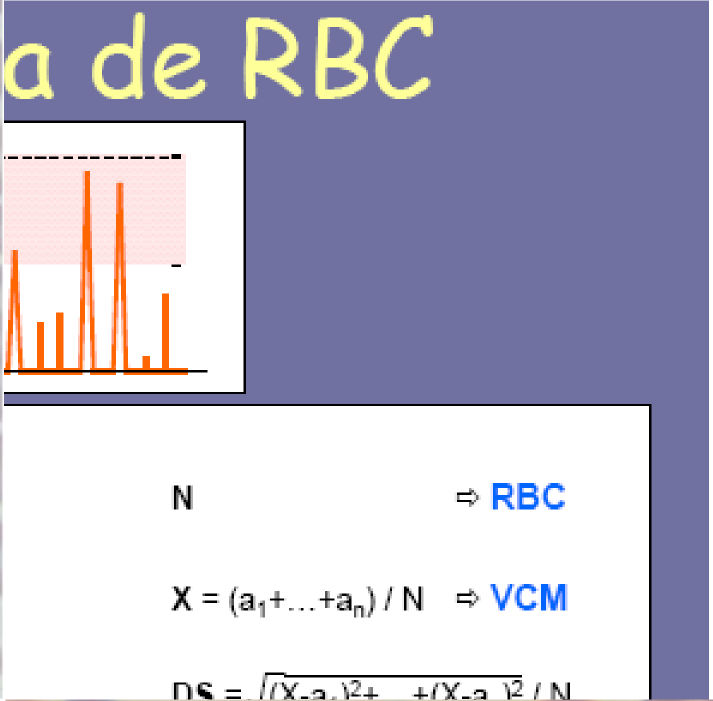
RBC extended parameters and Reticulocyte Hb

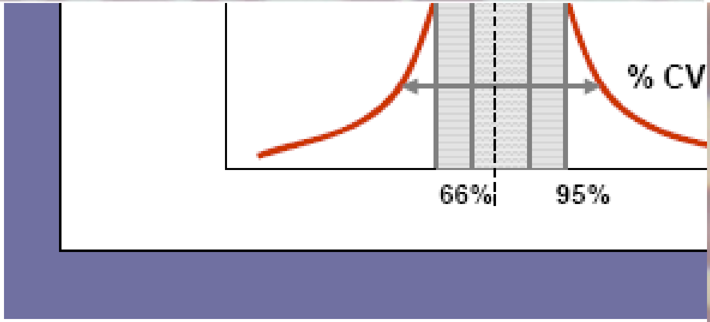
Hypochromic RBC	**Hypo (%)**	**Siemens**
Reticulocyte Hb	**CHr (pg)**	**Siemens**
Hypochromic RBC	**%HPO (%)**	**Abbott**
Mean Reticulocyte Hb	**MCHr (pg)**	**Abbott**
Hypochromic RBC	**Hypo He (%)**	**Sysmex**
Equivalent Reticulocyte Hb	**Ret He (pg)**	**Sysmex**
Low Hb Density	**LHD (%)**	**Beckman Coulter**
Red Cell Size Factor	**RSf (fL)**	**Beckman Coulter**
Reticulocyte Hb Content	**RHCc (pg)**	**Horiba ABX**
Reticulocyte Hb Expresion	**RHE (pg)**	**Mindray**
Hypochromic RBC	**HYPO (%)**	**Mindray**

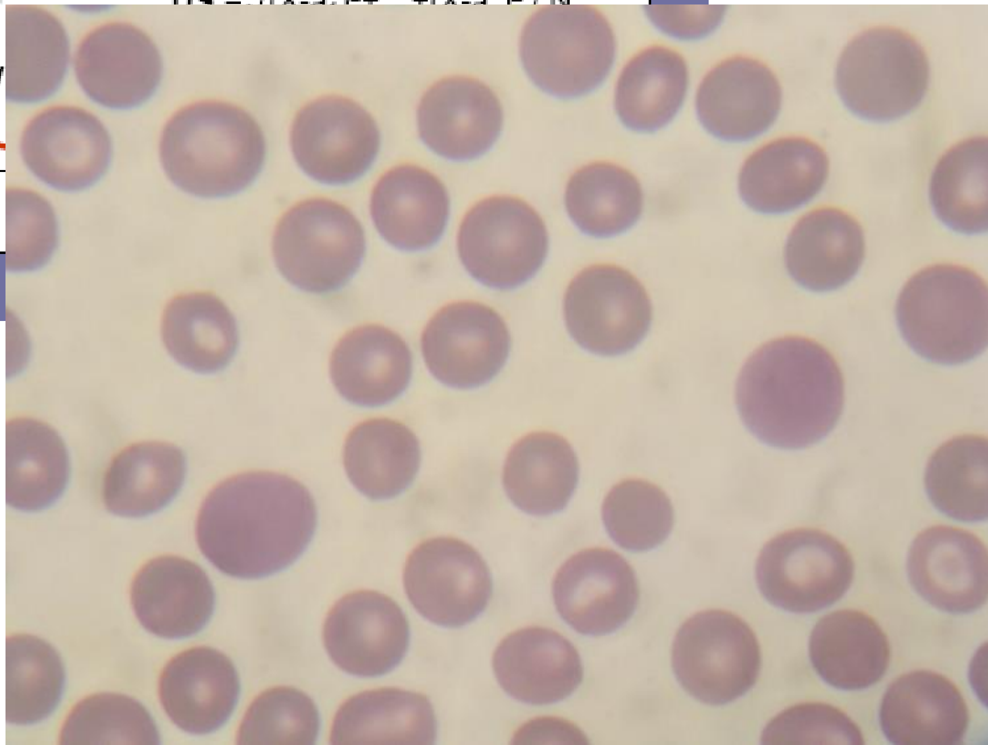
Urrechaga E, et al. Biomarkers of hypochromia: the contemporary assessment of Iron status and erythropoiesis. Journal of Biomedicine and Biotechnology 2013http://dx.doi.org/10.1155/2013/603786

Archer NM, Brugnara C . Crit Rev Clin Lab Sci, 2015; 52(5): 256–272

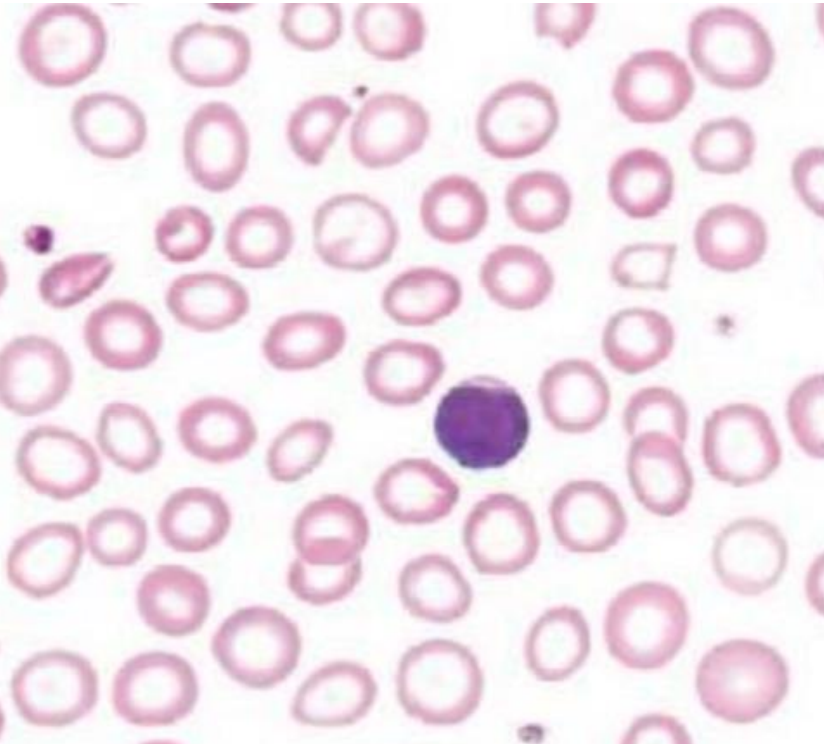


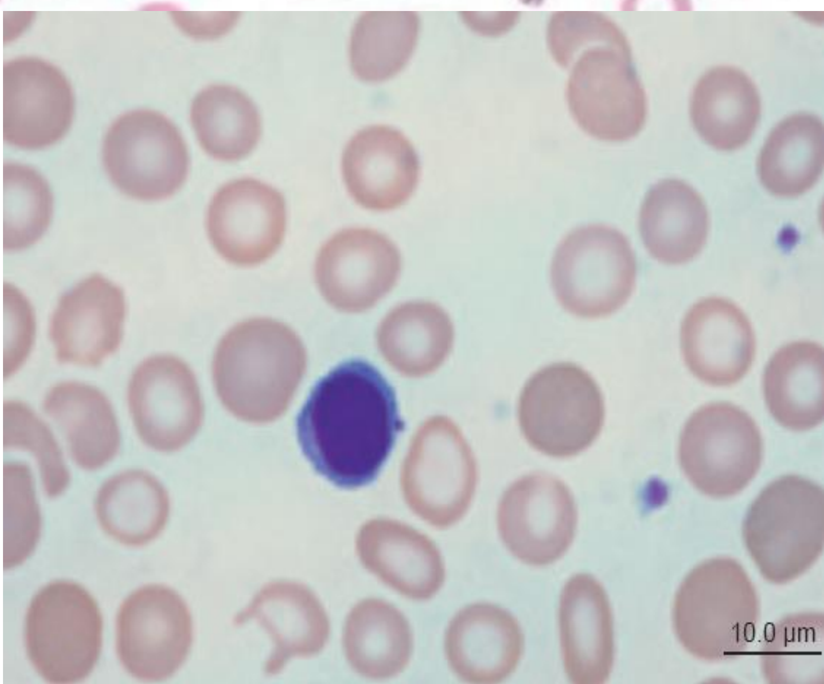






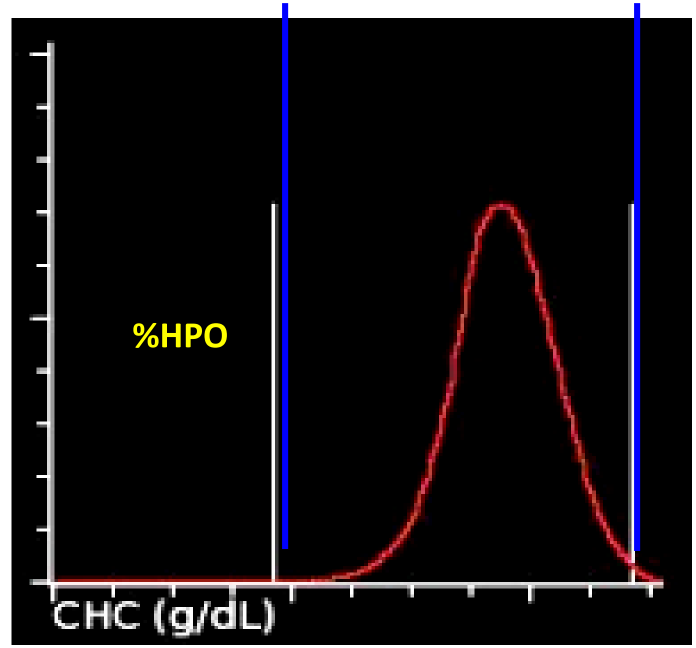
Bessman JD et al. Improved classification of anemias by MCV and RDW
Am J Clin Pathol 1983; 80: 322-326





Extended RBC parameters

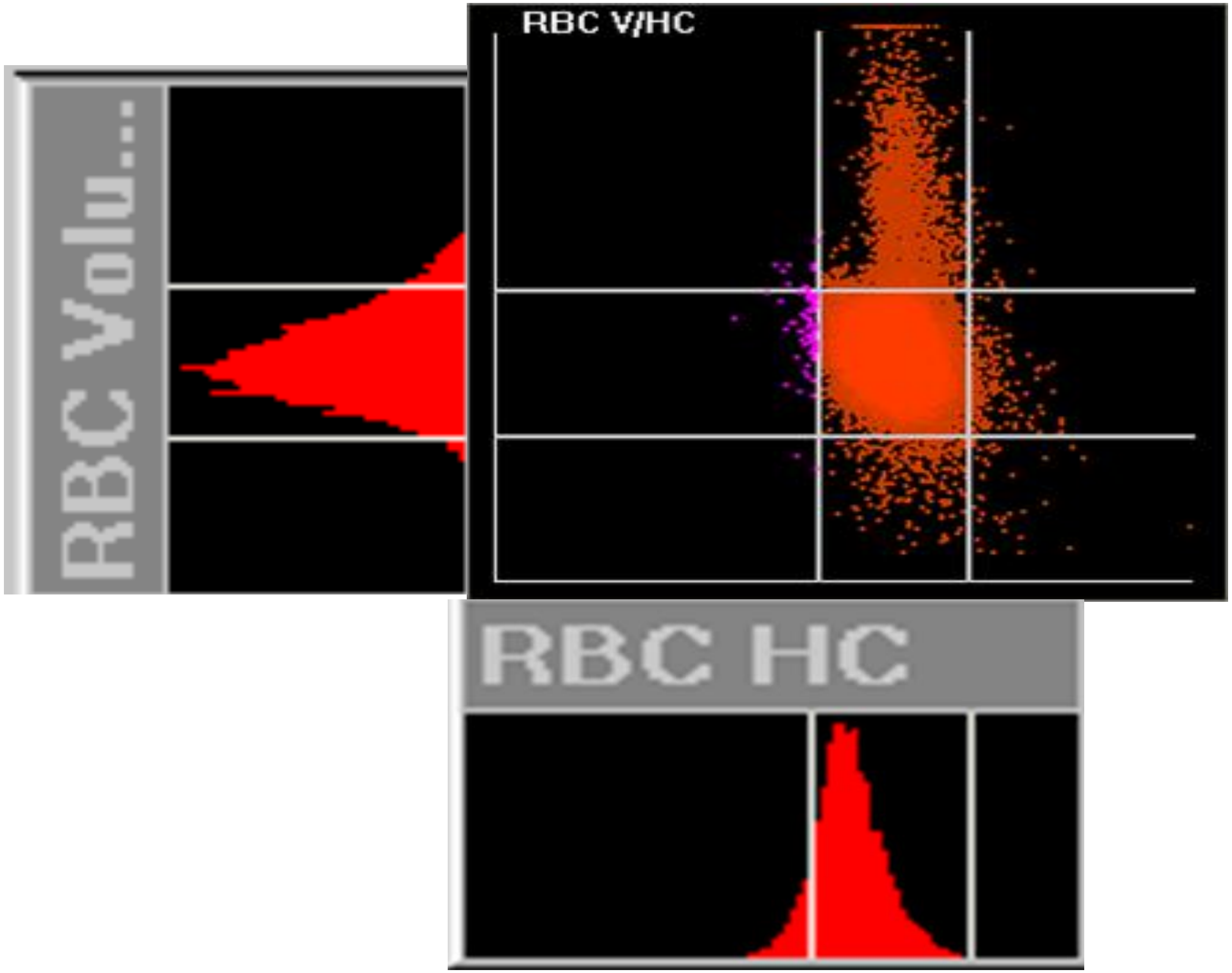
nacrocytic RBC)
ne < 60 fL and > 120 fL



), %HPR (hypochromic & hyperchromic RBC)
ion of RBC with CHC < 280 g/L and > 410 g/L

more sensitive marker because small changes in the number of RBC with inadequate Hb can be accurately measured

RBC Extended parameter graphics
RBC cytogram Mie Map



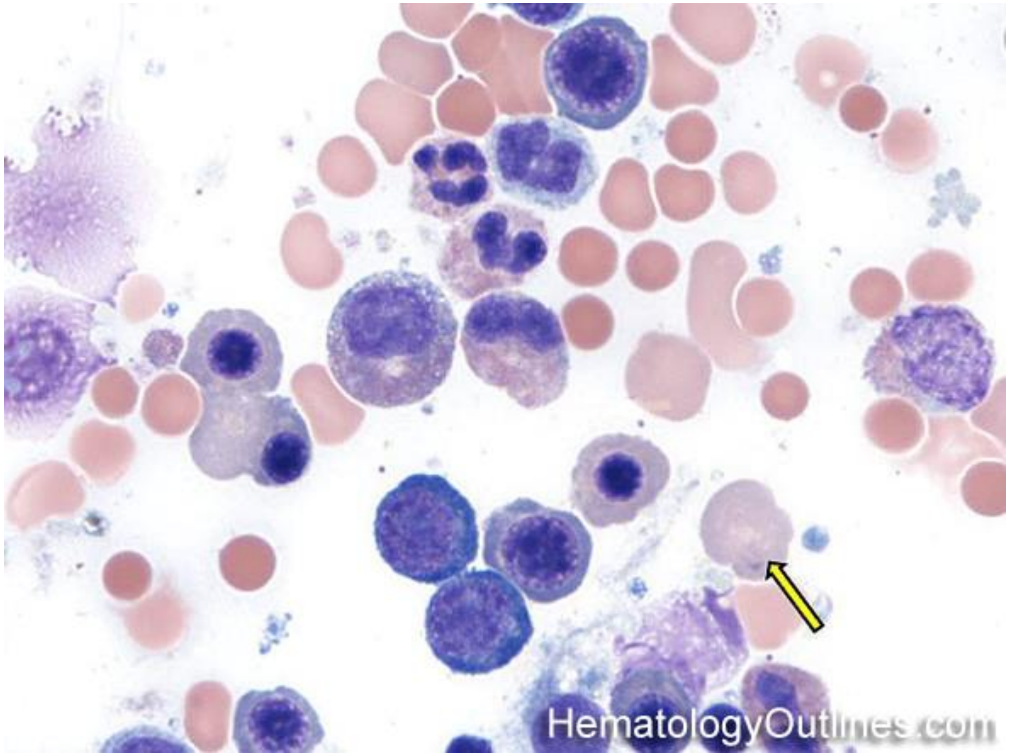
RBC classified by volume & Hb concentration

Reticulocyte & derived parameters

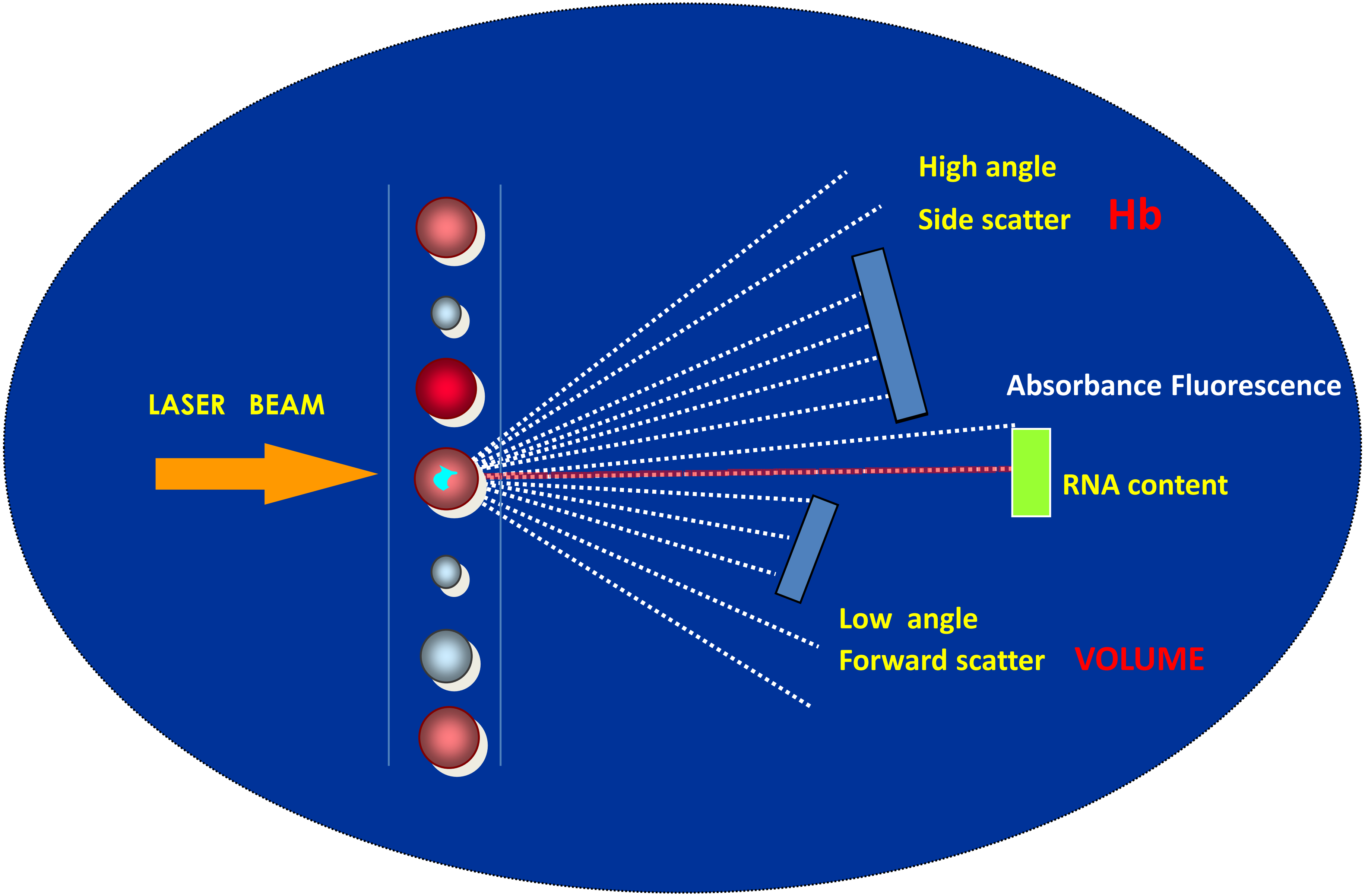
Reticulocytes are immature erythrocytes precursors and their conversion to erythrocytes takes 3 to 4 days, first in the bone marrow and in the last 1-2 days in the circulation

Circulating reticulocytes do not synthesize hemoglobin, unlike reticulocytes in the bone marrow

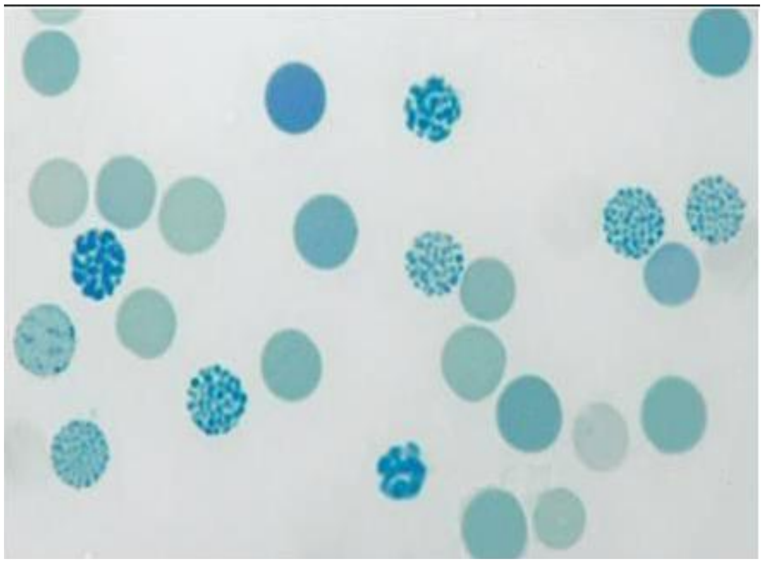
The measurement of their Hb content reflects the amount of iron immediately available for erythropoiesis and provides direct information on iron-deficient erythropoiesis over the previous period of 3-4 days

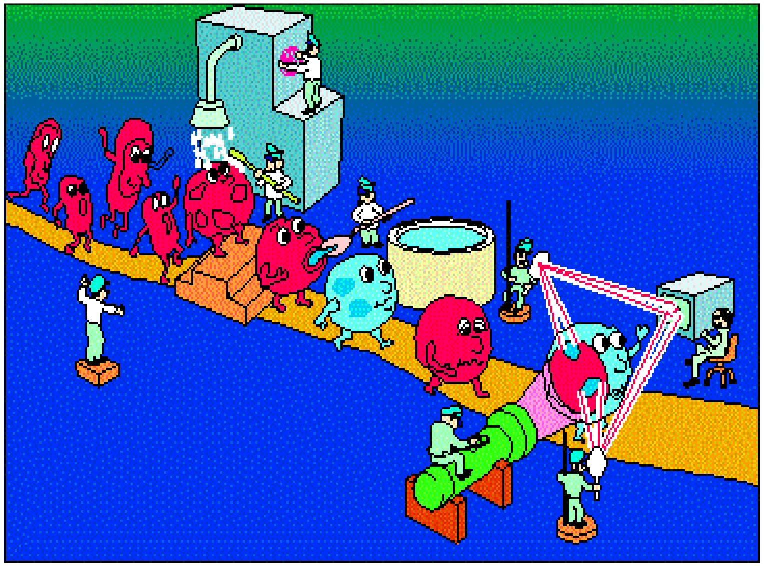


Flow Cytometry



Reticulocyte derived parameters





Reticulocyte count is a quantitation of bone marrow activity, and the base to classify anemia as regenerative

Reticulocyte Volume and Hb content , describe the quality of erythropoiesis
adequate supply of nutrients for Hb synthesis
early detection of negative iron balance requirements/suppy

Reticulocyte Hemoglobin Expression-RHE

RET-Reticulocyte:

the most newly released precursor cells from the peripheral blood of the bone marrow, and short life cycle, and it can respond to the hematopoiesis of bone marrow in time.

RHE(Reticulocyte Hemoglobin Expression)：

Early index of functional iron deficiency / iron deficiency erythrohemopoiesis / iron deficiency anemia;

RHE gradually decreases with the increase of iron deficiency, and is better than HGB/MCV for detection of iron deficiency, especially pure iron deficiency in underaged females.

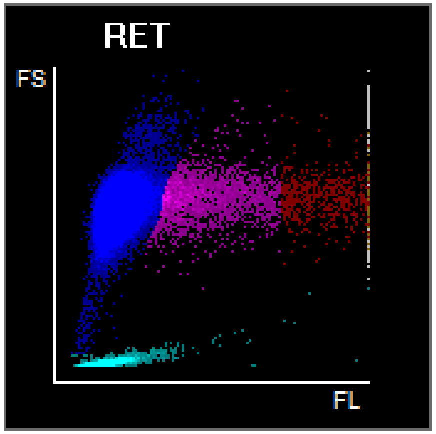
RHE decreases：

α-thalassemia/β-thalassemia / chronic anemia

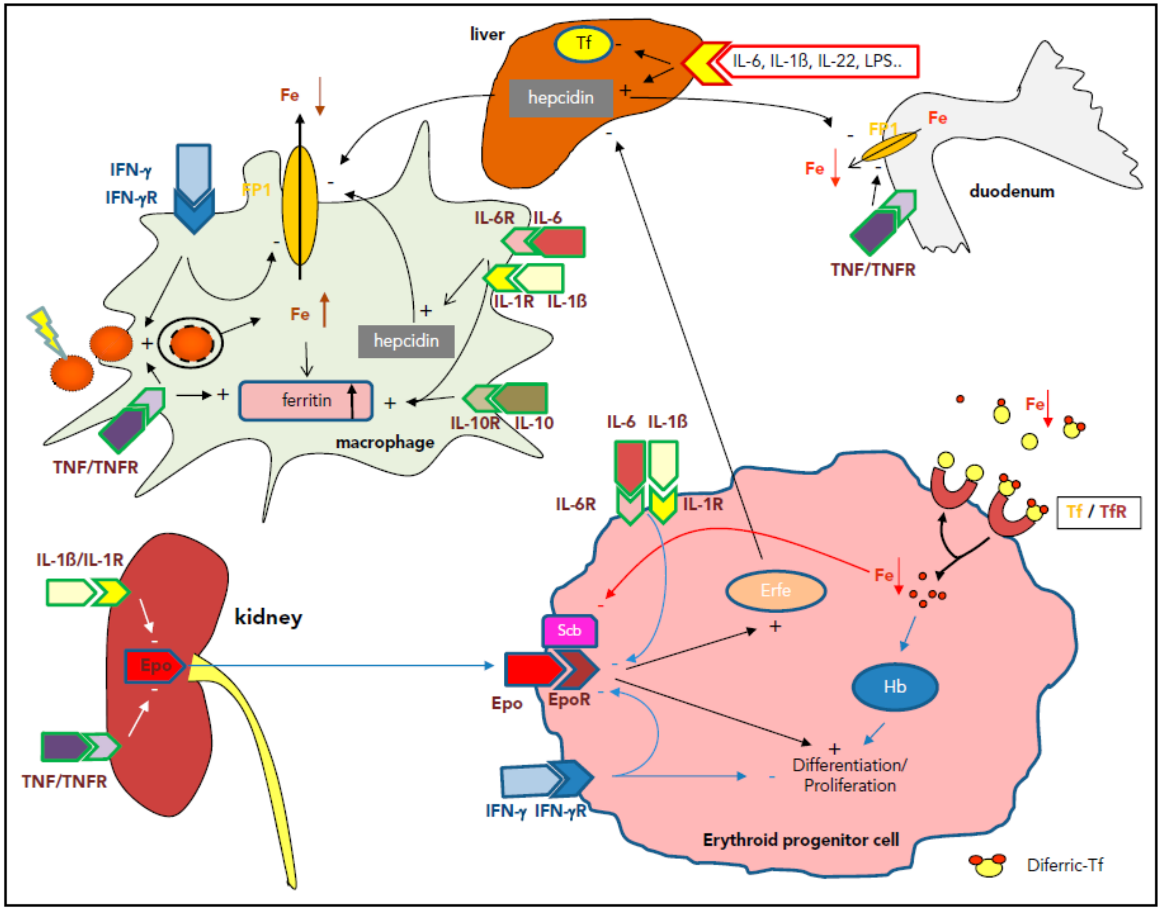
RHE increases：

megalocytic anemia: such as folic acid and vitamin B12 deficiency

influence factors: recent blood transfusion / iron therapy etc.



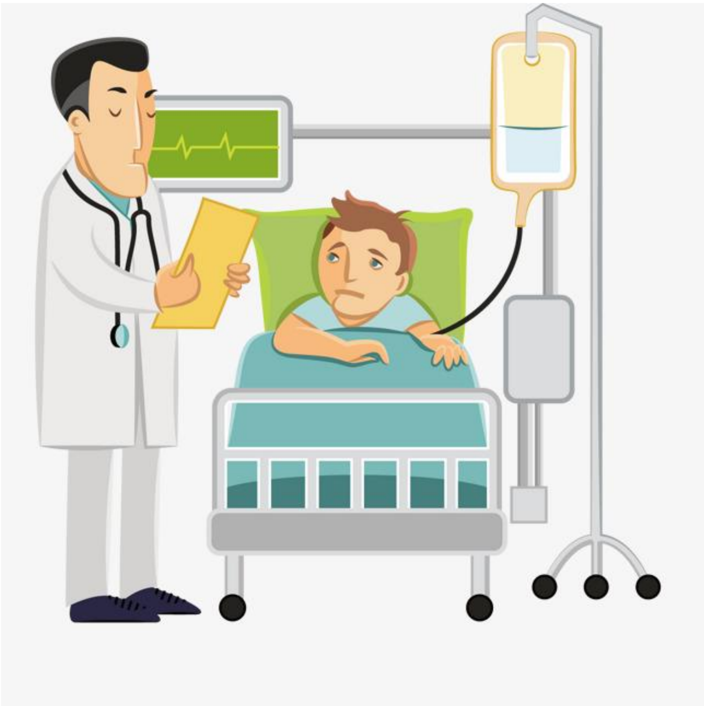
Anemia of chronic disease   Functional Iron deficiency



Macrophages that normally recycle iron, as a consequence of inflammation, sequester it
Consequently, decreased serum iron is available for erythropoiesis

Functional Iron deficiency : Lack of availability of pool iron storage in relation to the demands, to maintain a erythropoiesis level adapted

Weiss G, Ganz T, Goodnough LT. Anemia of inflammation. Blood. 2019;133(1):40-50



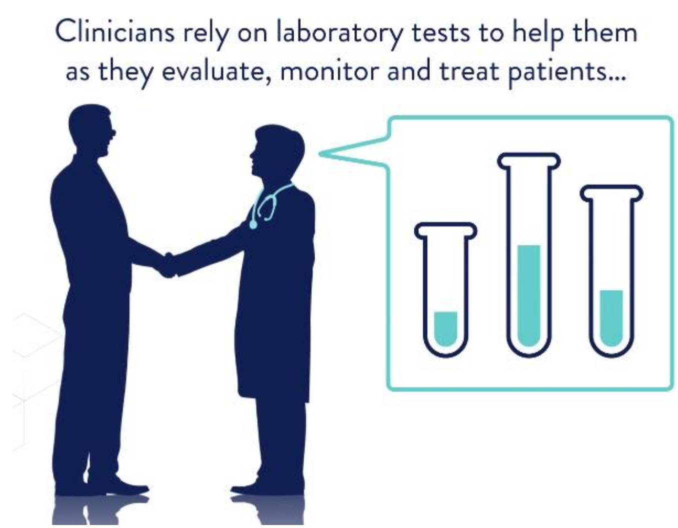
The Clinician's Need for Reliable Laboratory Tests

Recombinant human erythropoietin (rHuEpo) for the treatment of patients with anemia related CKD has been available since 1989

Monitoring erythropoietin treated patients' iron status is important to detect iron deficiency and avoid the adverse effects of iron medication

To evaluate iron available for erythropoiesis:

transferrin saturation
percentage of hypochromic red cells (Siemens) **6 %**
reticulocyte hemoglobin content (Siemens) **30 pg**



Revised European best practice guidelines for the management of anaemia in patients with chronic renal failure.
Nephrol Dial Transplant 2004; 19 (suppl 2): 1-47. Guideline 1.2

Thomas DW, Hinchliffe RF, Briggs C, Macdougall IC, Littlewood T, Cavill I

Guideline for the laboratory diagnosis of functional iron deficiency

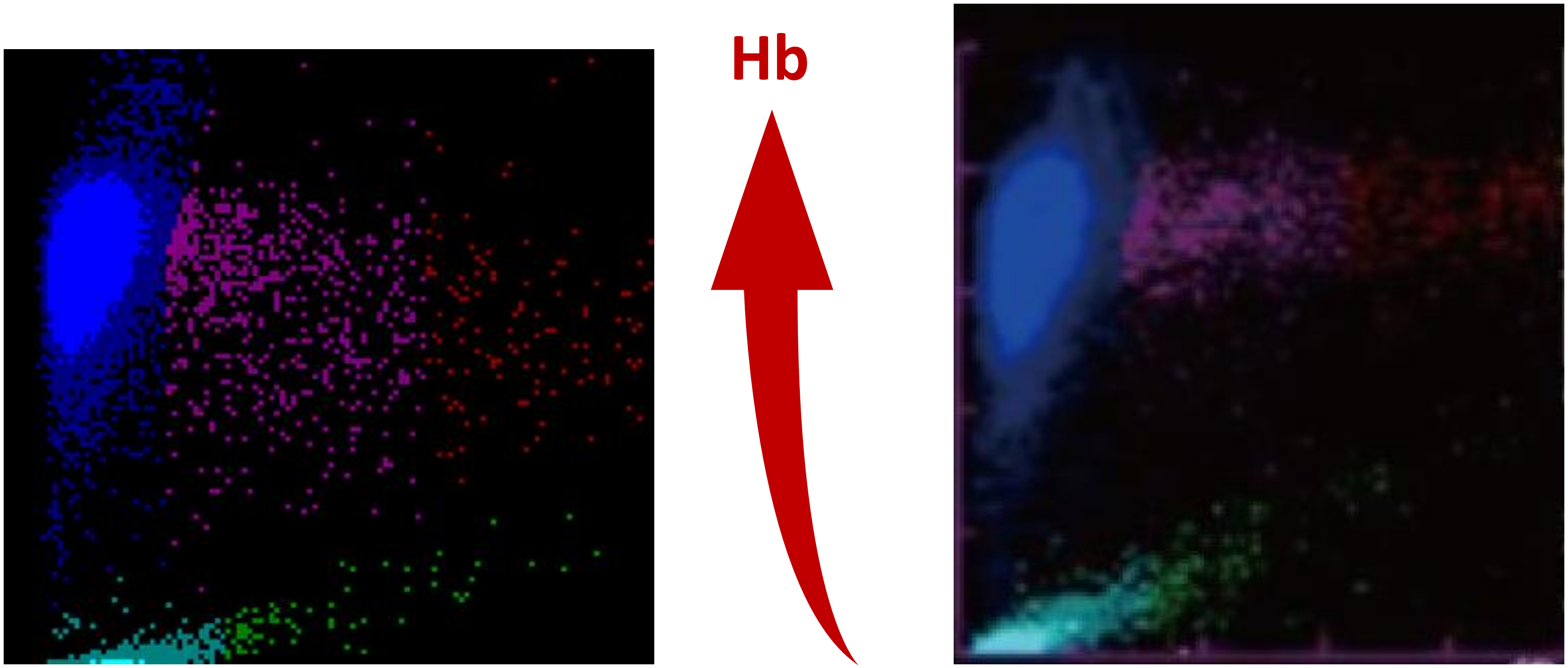
Br J Hematol 2013; 161:639-648

- **MCH, MCV 1B**
- ✓ **% Hypochromic RBC 1B**
- ✓ **Reticulocyte Hb 1B**
- **Protoporphyirin Zn 1B**
- **Bone Marrow 1B**
- **Ferritin 1 A 1 B**
- **sTfR 1A**
- **Sat transferria 1 A 1 B**
- **Erythropoyetin 1 A**
- **Hepcidin UE**

Recommendation

- The %HRC is the best-established variable for the identification of functional iron deficiency (FID) and thus has the greatest level of evidence (Tessitore *et al*, 2001). CHr is the next most established option. Both tests have limitations in terms of sample stability or equipment availability. Other parameters may be as good but there is no evidence that they are any better, and generally there is less evidence for newer red cell and reticulocyte parameters.
- A CHr value <29 pg predicts IRE in patients with iron deficiency anaemia, FID and those receiving ESA therapy. A Ret-He value <25 pg predicts FID in those receiving ESA therapy. Among reticulocyte variables, a Ret-He value <30·6 pg appears to be the best predictive value for response to intravenous iron in CKD patients on haemodialysis.

The evolution of erythropoiesis in response to therapy



Aids clinicians in decision making
Automated, cheap , fast
Reliable measurement

Turning technology into better caring

Clinical utility of Reticulocyte Hemoglobin and Hypochromic erythrocytes reported by Mindray BC6800 Plus Hematology Analyzer in the study of erythropoiesis

May 19-23
BARCELONA
EUROMEDLAB 2019

330 samples collected in K2EDTA anti-coagulant were run sequentially on both Sysmex XN-20 and Mindray BC 6800 Plus Analyzers

The scope of the pathology included a variety of diseases representative of the daily workload:
80 healthy subjects,
84 iron deficiency anemia IDA
87 anemia of chronic disease ACD
79 thalassemia carriers

C reactive protein, S- Iron, Transferrin saturation, s-Ferritin, soluble transferrin receptor (sTfR)

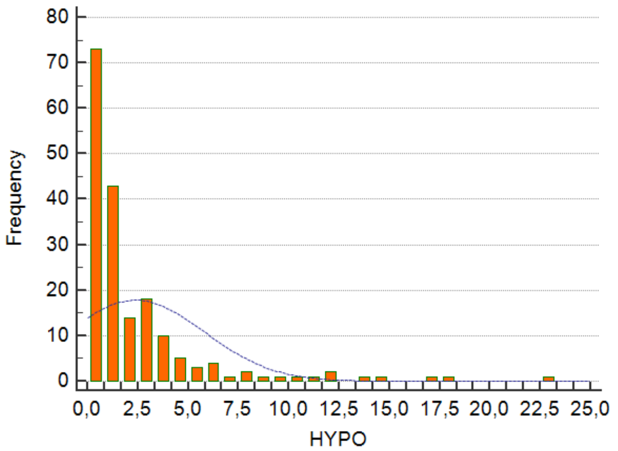
Kolmogorov-Smirnoff was used to verify normality

Correlation between CHr and Ret He was assessed with Spearman's coefficient ;
a polynomial equation for non-linear correlations was applied (HYPO and Hypo He)

ROC was used to assess the diagnostic performance of CHr, and HYPO for detecting iron deficient erythropoiesis.
Gold standard for low iron availability was sTfR >52 nmol/L.

Clinical utility of Reticulocyte Hemoglobin and Hypochromic erythrocytes reported by Mindray BC6800 Plus Analyzer in the study of erythropoiesis

Sweked distribution was proven for CHr , Ret-He, HYPO and Hypo He



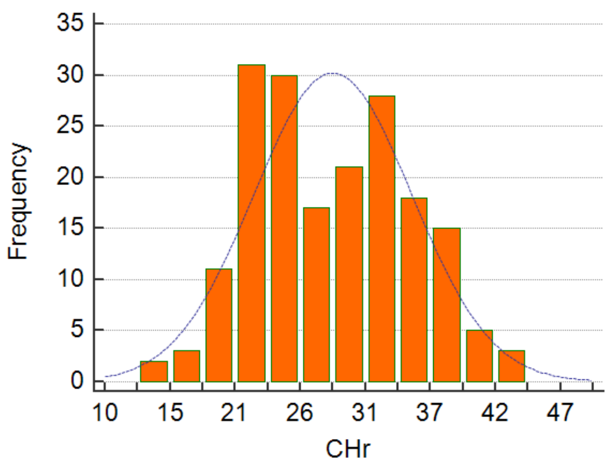
Median and 25-75th quartiles in healthy subjects

CHr 33.3 pg, 32.0-34.5 pg; HYPO 0.1 % 0.1-0.3%

Ret He 30.0 pg 29.3-32.3 pg Hypo He 0.2 % 0.1-0.6%

Whole range RetHe 20.6-42.5 pg, CHr 25.0-46.1 pg

HypoHe 0.1-14 % %Hypo 0.1-30 %



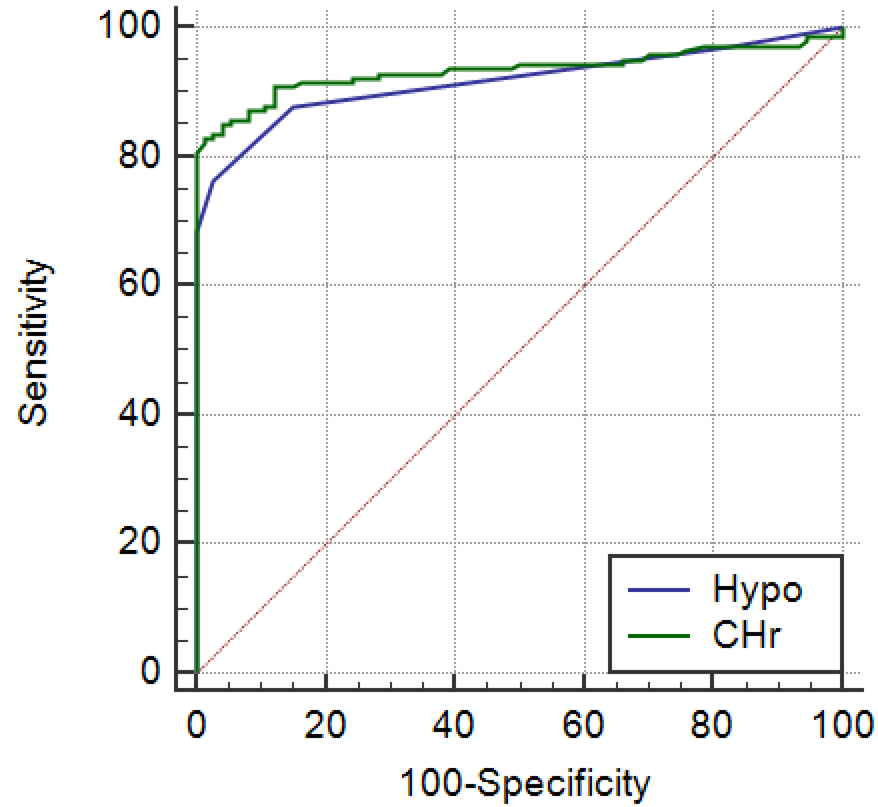
Linear correlation Ret-He and CHr

y=1.054x-1.86. (95%CI -5.2-1.7 slope; .95-1.1 intercept)

Correlation between HypoHe and HYPO can be described by a 2nd degree polynomial equation

$$y=0.0082x^2 +0.765x+0.446$$

We aimed to study the diagnostic performance of these parameters of hemoglobinization of red cells in the detection of functional iron deficiency

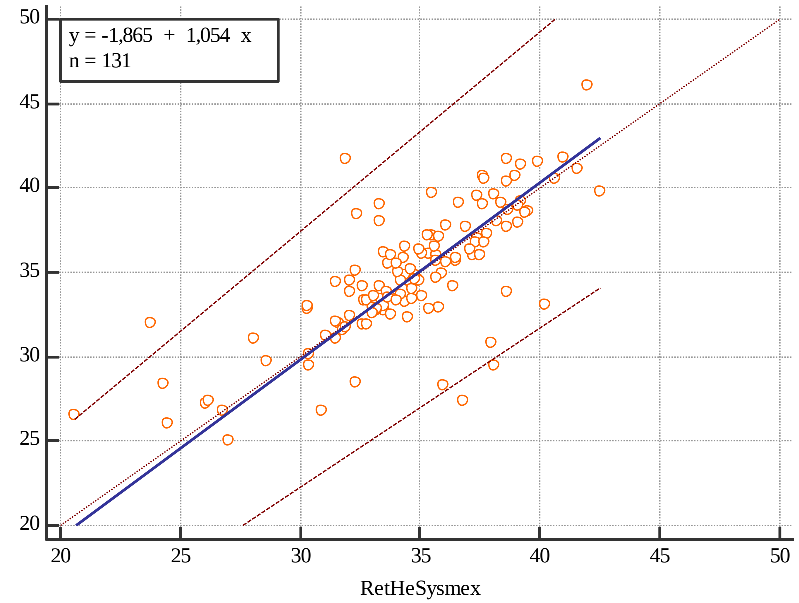


	AUC	95%CI	CutOff	Sensitivity%	Specificity%
CHr	0.934	0.892–0.963	29.0 pg	82.9	98.6
Hypo	0.918	0.872–0.951	6.0 %	76.4	97.3

RetHe and CHr are directly comparable.

- ✓ Technological advances in automated full blood count analysers allows the hemoglobin content of individual red cells to be measured by flow cytometry, so it is possible to calculate the number of individual red cells with low hemoglobin content

- ✓ CHr is a reliable marker to provide an estimation of the iron available for erythropoiesis improving the evaluation of iron requirements identifying iron deficient erythropoiesis



Reticulocyte Hemoglobin (CHr) reported by Mindray BC6800 Plus in the study of anemia

416 samples were run on Mindray BC 6800 Plus analyzer

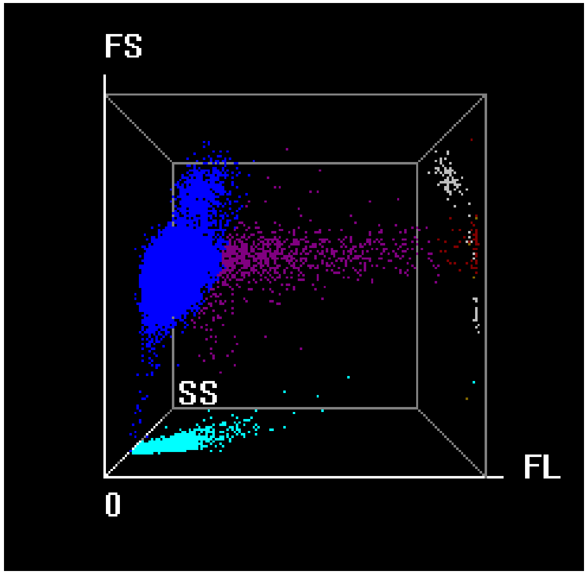
The scope of the pathology included a variety of diseases representative of the daily workload:

80 healthy subjects

202 microcytic anemia: thalassemia carriers and IDA

91 normocytic anemia : hematology malignancies and ACD

37 macrocytic anemia :lack of vitamin B12 or folate and MDS



C reactive protein,

Serum iron, t

Transferrin saturation,

Ferritin

Soluble transferrin receptor (sTfR)

Folate/vitamin B12

Kolmogorov-Smirnoff was used to verify normal distribution of data
Differences among groups were assessed using analysis of variance, considering $P < 0.05$ to be significant. For post hoc comparisons of outcomes between each pair of groups Scheffé correction was applied.

Correlation coefficient between erythrocyte indices and CHr was calculated using the method of Pearson

Receiver operating characteristic analysis was used to assess the diagnostic performance of CHr for detecting iron deficient erythropoiesis. Gold standard for iron deficiency was sTfR >52 nmol/L.

Reticulocyte Hemoglobin (CHr) reported by Mindray BC6800 Plus in the study of anemia

Healthy subjects CHr 33.2 pg, 28.9-37.5 pg

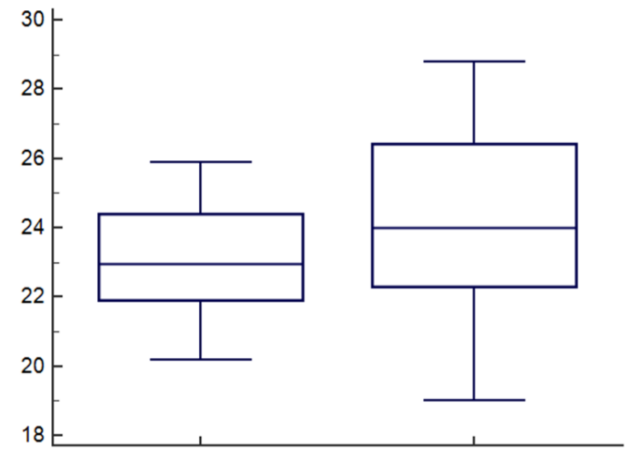
Microcytic anemia mean 23.7 pg, SD 2.75 pg

Normocytic anemia Mean 32.3 pg, SD 3.84 pg

Macrocytic anemia Mean 37.9 pg, SD 4.41 pg

In the microcytic group, the values in patients with IDA (CHr mean 23.8 pg, SD 1.7pg) and thalassemia carries (CHr mean 23.1 pg, SD 3.0 pg) were not significantly different P=0.0756.

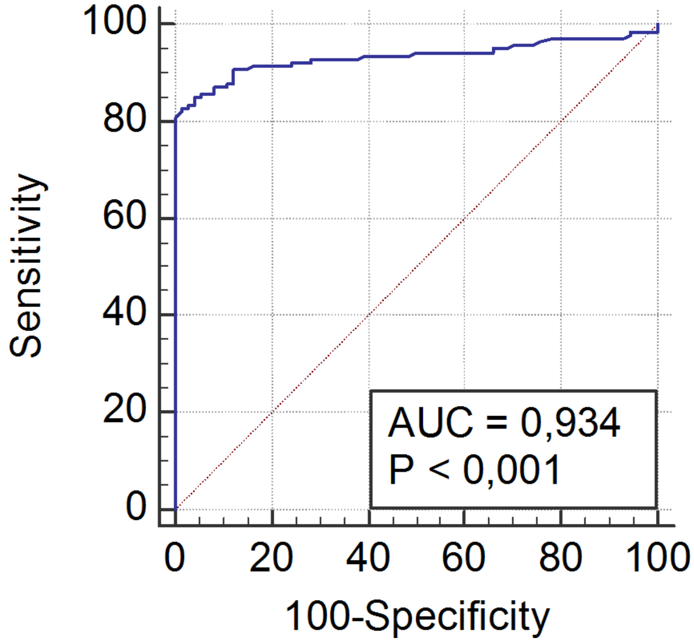
- Patients with restricted erythropoiesis, due to lack of iron or globin, had simmilar low values

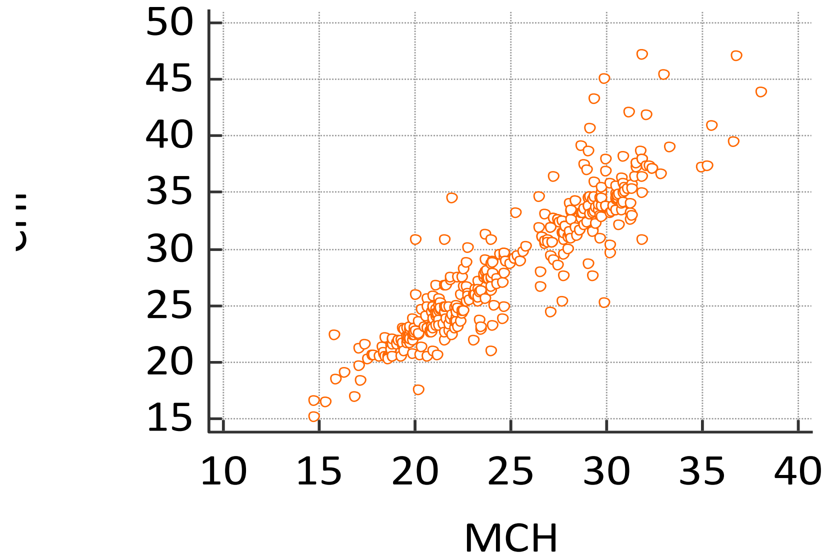


Values over the reference range in the macrocytic group is not related to iron status, reflects the megaloblastosis

Reticulocyte Hemoglobin (CHr) reported by Mindray BC6800 Plus in the study of anemia

Correlation MCH/CHr $R^2=0.9232$
$P<0.0001$ (95%CI 0.906-0.936)





	AUC	95%CI	CutOff	Sensitivity	Specificity
CHr	0.934	0.906 - 0.936	29.0 pg	82.9 %	98.6 %

Reticulocyte Hemoglobin (CHr) reported by Mindray BC6800 Plus in the study of anemia

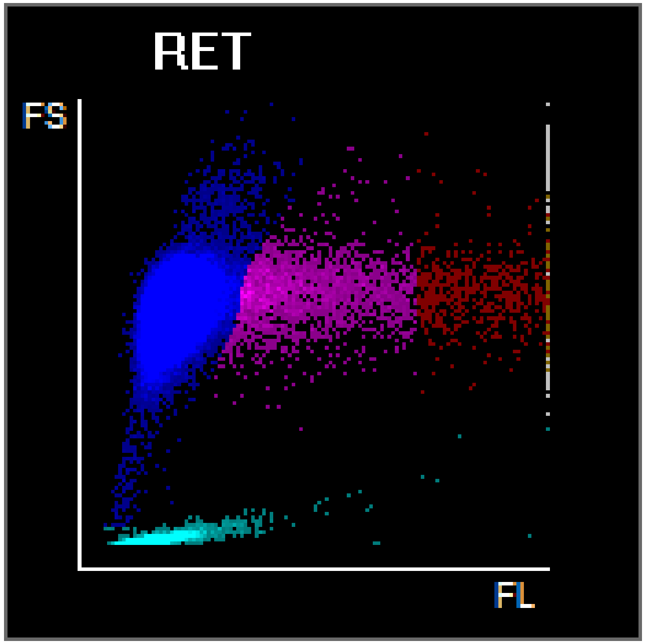
- ✓ Disturbances in erythropoiesis and iron metabolism may occur in many patients, the challenge is to identify these patients as early as possible

- ✓ Technological advances in automated full blood count analysers allows the hemoglobin content of individual red cells to be measured by flow cytometry, so it is possible to calculate the number of individual red cells with low hemoglobin content

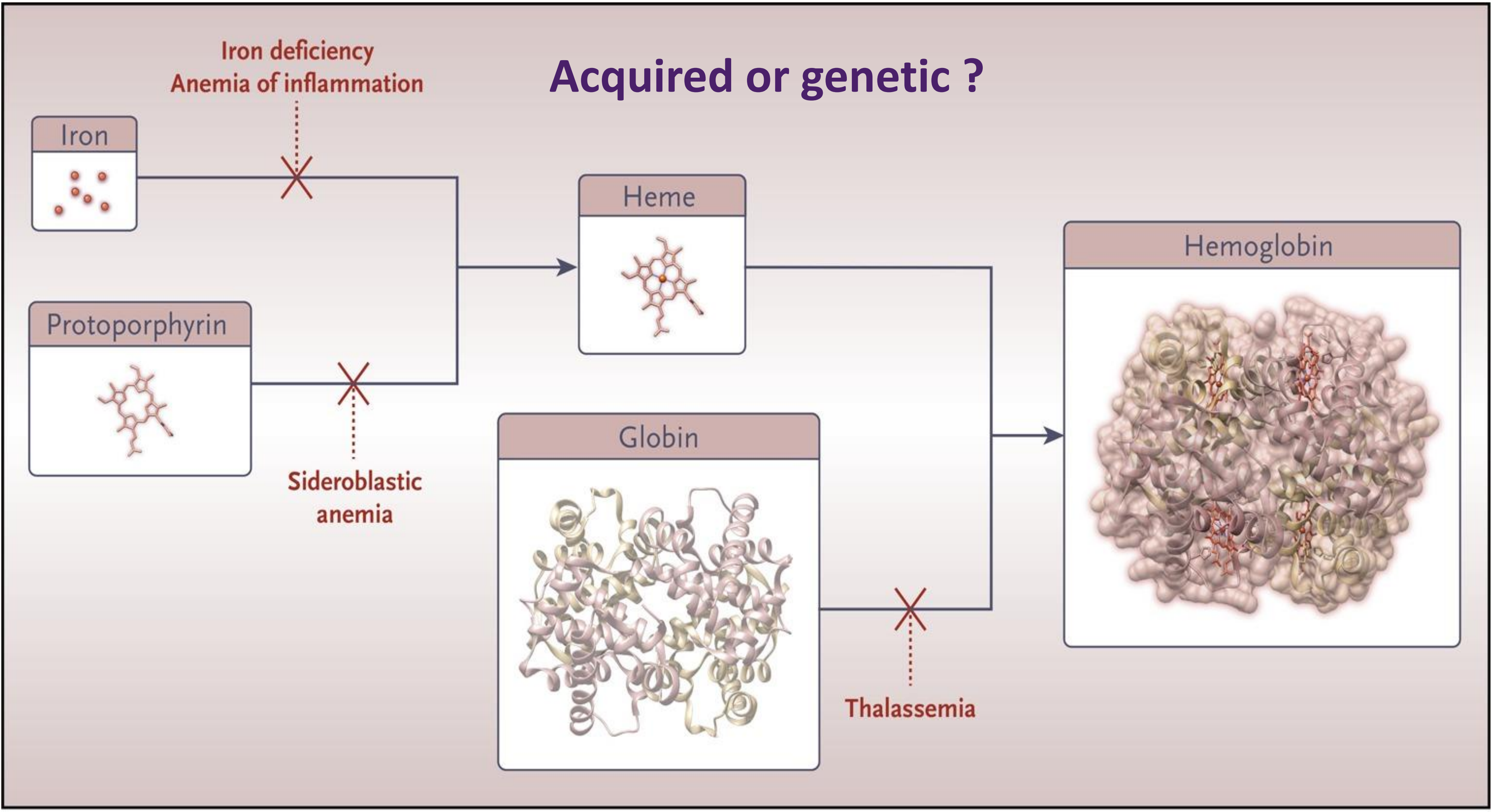
- ✓ CHr provides a sensitive method for quantifying the hemoglobinization of reticulocytes

- ✓ It is a reliable marker to identify iron deficient erythropoiesis, CHr may allow the complete scope of disorders of iron metabolism to be identified quickly and managed

ISLH 2019
Vancouver, Canada

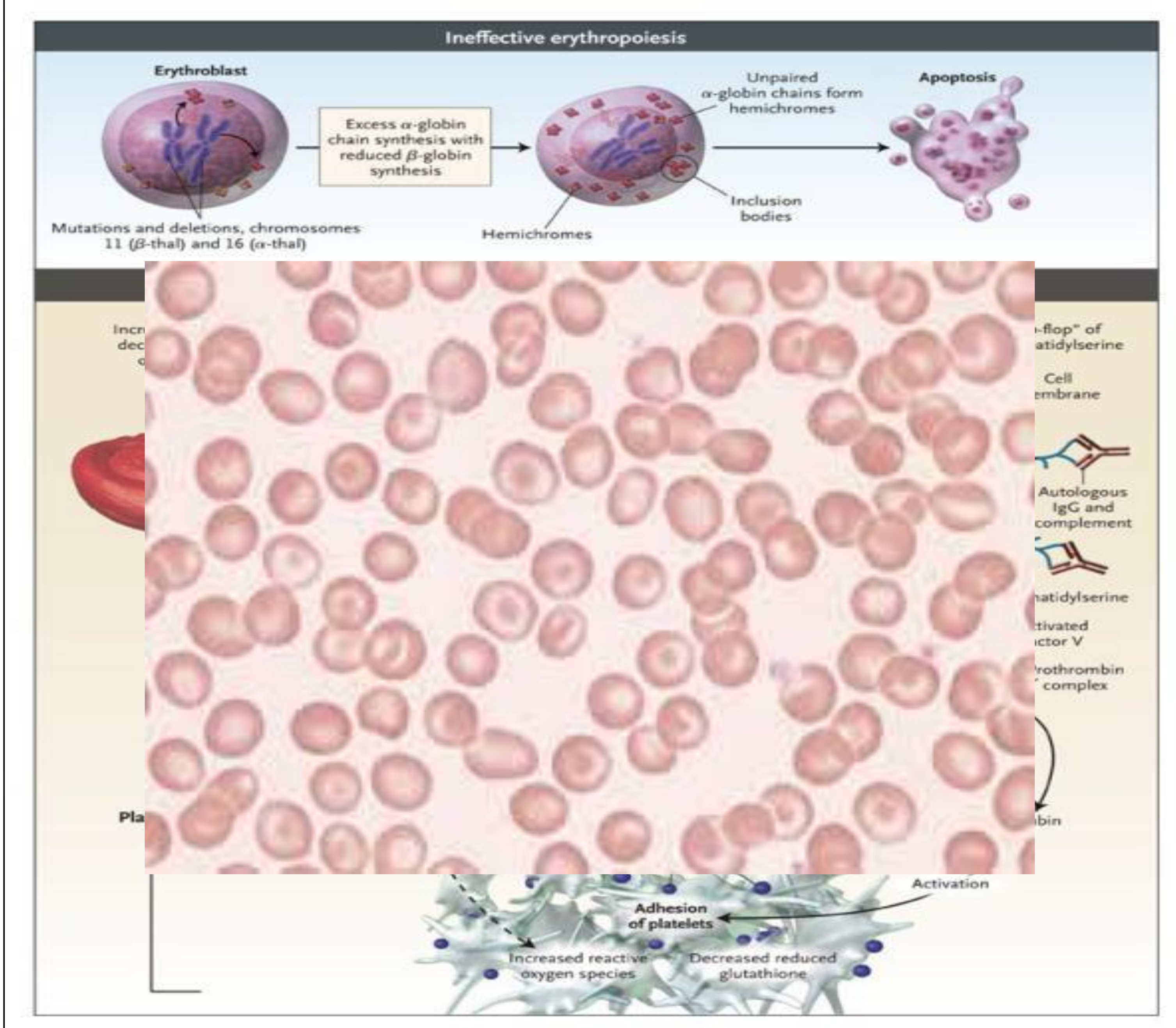


Disorders Characterized by Microcytosis



DeLoughery TG. N Engl J Med 2014;371:1324-1331

The NEW ENGLAND JOURNAL of MEDICINE

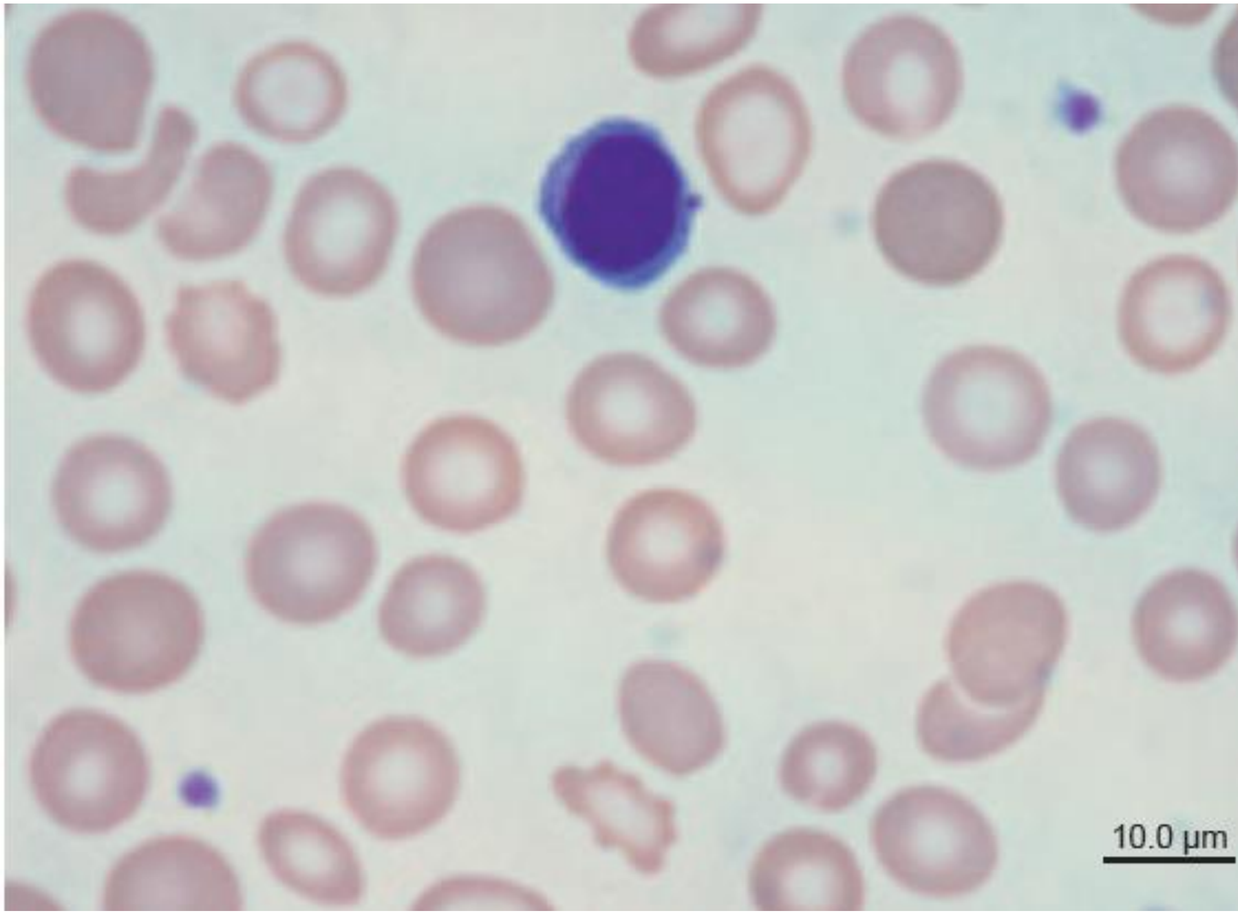


Rund & Rachmilewitz **Beta-thalassemia.** New England Journal of Medicine 2005: 353(11):1135-46

SPECTRUM OF IRON DEFICIENCY

STORAGE
(eg. liver, R

TRANSPO
FUNCTIO
(eg. HB, my



EFICIENCY
AEMIA

Example o

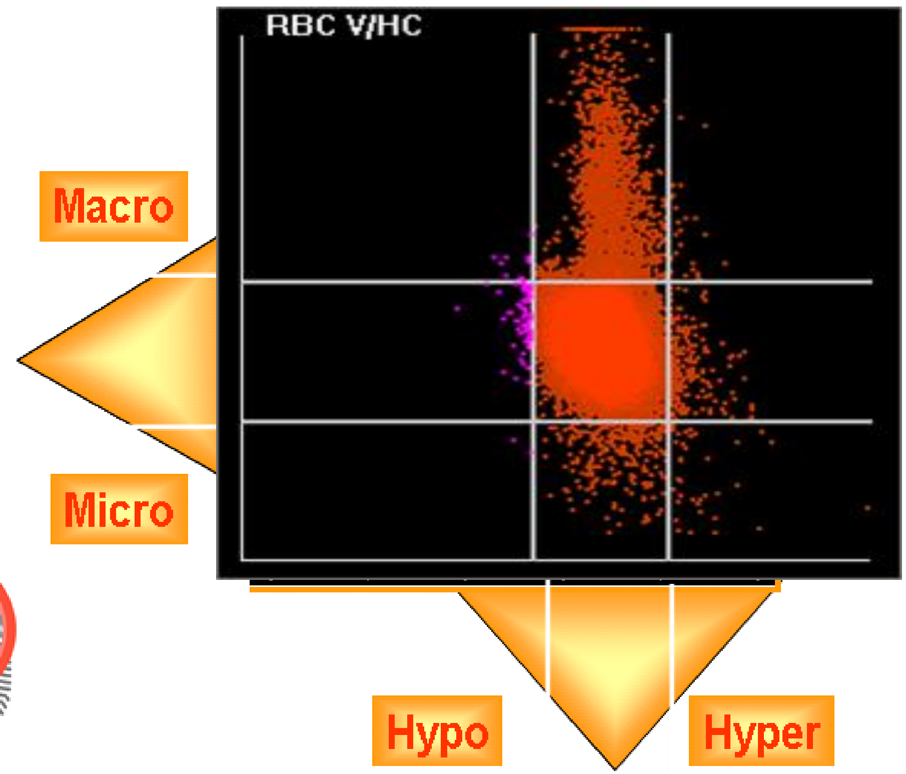
Serum fer	<15
Transferri	<15
Haemoglol	:120
Haemoglol	:130

Volume/Hb Concentration cytogram Mie Map

Screening of Hb disorders must rely on inexpensive methods

"Suspicious" samples can be selected to confirm diagnosis : allow an efficient use of the resources

Improvement the Laboratory workflow and efficiency : lean system of high throughput

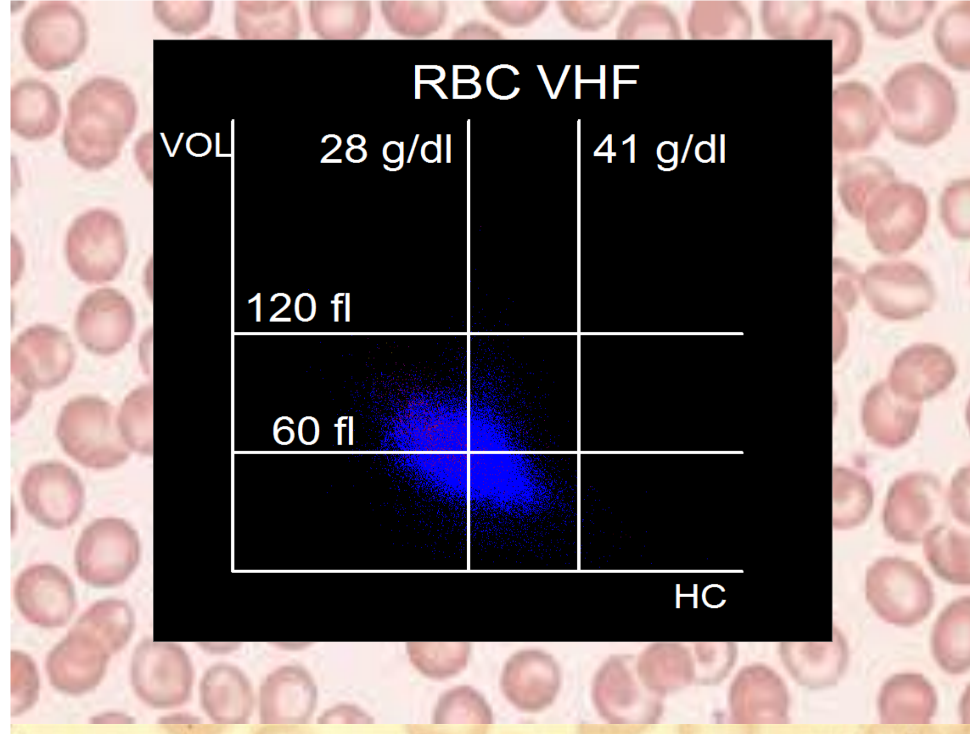


CBC Appropriate screening, detection of carriers, and counsel of couples

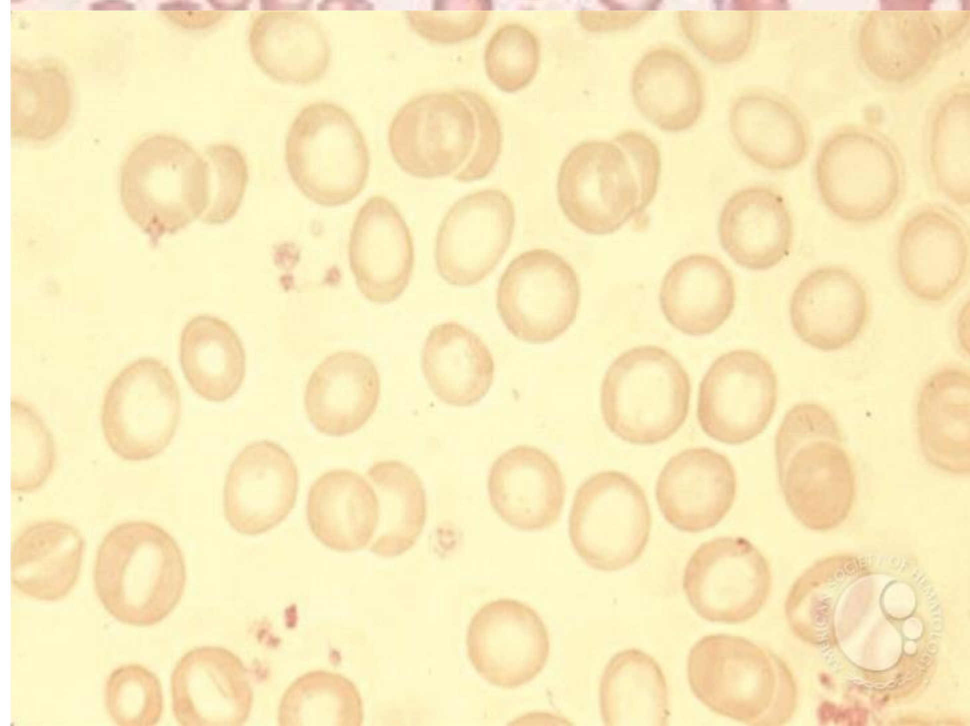
Clinicians reach a prompt accurate diagnosis : reduces unnecessary diagnostic testing and avoid inappropriate treatment

Turning technology into better caring

RBC	5.34	10^{12}/L
Hb	124	g/L
MCV	74.3	fL
MCH	23.2	pg
MCHC	312	g/L
RDW	16.4	%



RBC	5.03	10^{12}/L
Hb	118	g/L
MCV	75.1	fL
MCH	22.7	pg
MCHC	301	g/L
RDW	17.8	%



Erythrocyte Indices
differential diagnosis of microcytic anemia

Indices	Year	IDA	β Thalassemia
Mentzer = MCV / RBC	1973	> 13	< 13
Srivastava = MCH / RBC	1973	> 3.8	< 3.8
England & Fraser = MCV - RBC - 5*Hb - 3.4	1976	>0	<0
Ricerca = RDW / RBC	1987	> 4.4	< 4.4
Green & King = MCV^2 * RDW / 100* Hb	1989	> 65	< 65
MH ratio (Technicon) = %micro / %hypo	1992	<1	>1
Sirdah = MCV - RBC - 3*Hb	2008	>27	<27
MH ratio (Siemens)	2008	<3.4	>3.4
Ehsani = MCV - (10* RBC)	2009	> 15	< 15
ThalIndex=(0.615*MCV)+(0.518*MCH) +(0.446*RDW)	2012	>59	<59
MH ratio (Abbott)	2015	< 6.4	> 6.4

Johannes J.M.L. Hoffmann *, Eloisa Urrechaga, Urko Aguirre

Discriminant indices for distinguishing thalassemia and iron deficiency in patients with microcytic anemia: a meta-analysis

Clinical Chemistry & Laboratory Medicine 2015; 53(12):1883-94

Table 3: Diagnostic performance of the 12 discriminant indices, arranged in order of diagnostic odds ratio (DOR) with 95% confidence intervals (95% CI).

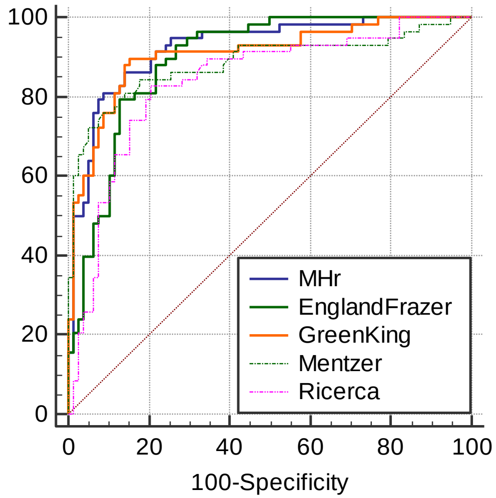
Discriminant index	DOR (95% CI)	PLR (95% CI)	NLR (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	AUC
M/H ratio	100.8 (39.6–256.3)	6.8 (4.8–9.8)	0.07 (0.03–0.2)	0.92 (0.87–0.98)	0.86 (0.81–0.91)	0.956
RBC	47.0 (29.5–74.9)	8.1 (5.8–11.4)	0.17 (0.13–0.22)	0.85 (0.80–0.88)	0.90 (0.86–0.93)	0.923
Sirdah	46.7 (23.4–92.9)	8.6 (4.8–15.5)	0.18 (0.12–0.27)	0.83 (0.75–0.89)	0.90 (0.83–0.95)	0.903
Ehsani	44.7 (26.8–74.7)	5.1 (3.7–7.0)	0.11 (0.10–0.18)	0.91 (0.85–0.94)	0.82 (0.76–0.87)	0.925
England and Fraser (E&F)	34.7 (25.0–48.2)	9.5 (7.2–12.6)	0.27 (0.23–0.32)	0.75 (0.70–0.79)	0.92 (0.90–0.94)	0.887
Green and King (G&K)	29.8 (18.5–47.8)	7.2 (5.2–10.0)	0.24 (0.2–0.3)	0.79 (0.73–0.83)	0.89 (0.85–0.92)	0.898
Jayabose (RDWI)	28.6 (17.8–45.9)	5.6 (4.4–7.1)	0.20 (0.14–0.27)	0.83 (0.78–0.88)	0.85 (0.81–0.88)	0.902
Mentzer	27.6 (20.7–36.6)	5.6 (4.6–6.8)	0.20 (0.17–0.24)	0.82 (0.79–0.86)	0.85 (0.82–0.88)	0.896
Shine and Lal (S&L)	15.7 (8.8–28.0)	1.6 (1.3–2.0)	0.10 (0.07–0.16)	0.96 (0.93–0.97)	0.41 (0.27–0.56)	0.885
Ricerca	15.6 (7.9–30.9)	2.0 (1.4–2.7)	0.12 (0.07–0.22)	0.93 (0.88–0.97)	0.52 (0.36–0.67)	0.850
Srivastava	15.0 (10.9–20.6)	4.1 (3.3–5.1)	0.28 (0.23–0.34)	0.78 (0.72–0.82)	0.81 (0.77–0.85)	0.850
Bessman (RDW)	6.8 (4.0–11.7)	5.1 (4.2–6.2)	0.21 (0.17–0.27)	0.62 (0.61–0.63)	0.66 (0.65–0.68)	0.778

AUC, area under the ROC curve; NLR, negative likelihood ratio; PLR, positive likelihood ratio. The higher DOR values, the better discriminatory test performance is present. Positive and negative likelihood ratios >10 and <0.1 indicate that the test generates strong evidence to rule in or rule out a thalassemia diagnosis, respectively.

DIFFERENTIAL DIAGNOSIS OF MICROCYTIC ANEMIA

	Year	IDA	Thalassemia
Mentzer = MCV / RBC	1973	> 13	< 13
England & Fraser = MCV - RBC - 5*Hb - 3.4	1976	> 0	< 0
Ricerca = RDW /RBC	1987	> 4.4	< 4.4
Green & King = MCV^2 * RDW / 100* Hb	1989	< 65	< 65

M/H ratio	AUC	95 % CI	Sen	Spec
	0.918	0.871 -0.966	90.3	81.1



Extended RBC parameters

- ✓ **Expand information at a cellular level**
- ✓ **Correlate with the pathophysiology of disease**

Improve the clinical relevant information

Quality of erythropoiesis Aids clinicians in

- ➢ **Assessing true iron status**
- ➢ **Detect Functional Iron Deficiency = patients who can benefit from therapy**
- ➢ **Differential diagnosis anemia genetic or acquired**

Ilginiz için teşekkürler

Thanks for your attention

Gracias por su atención

感谢您的关注