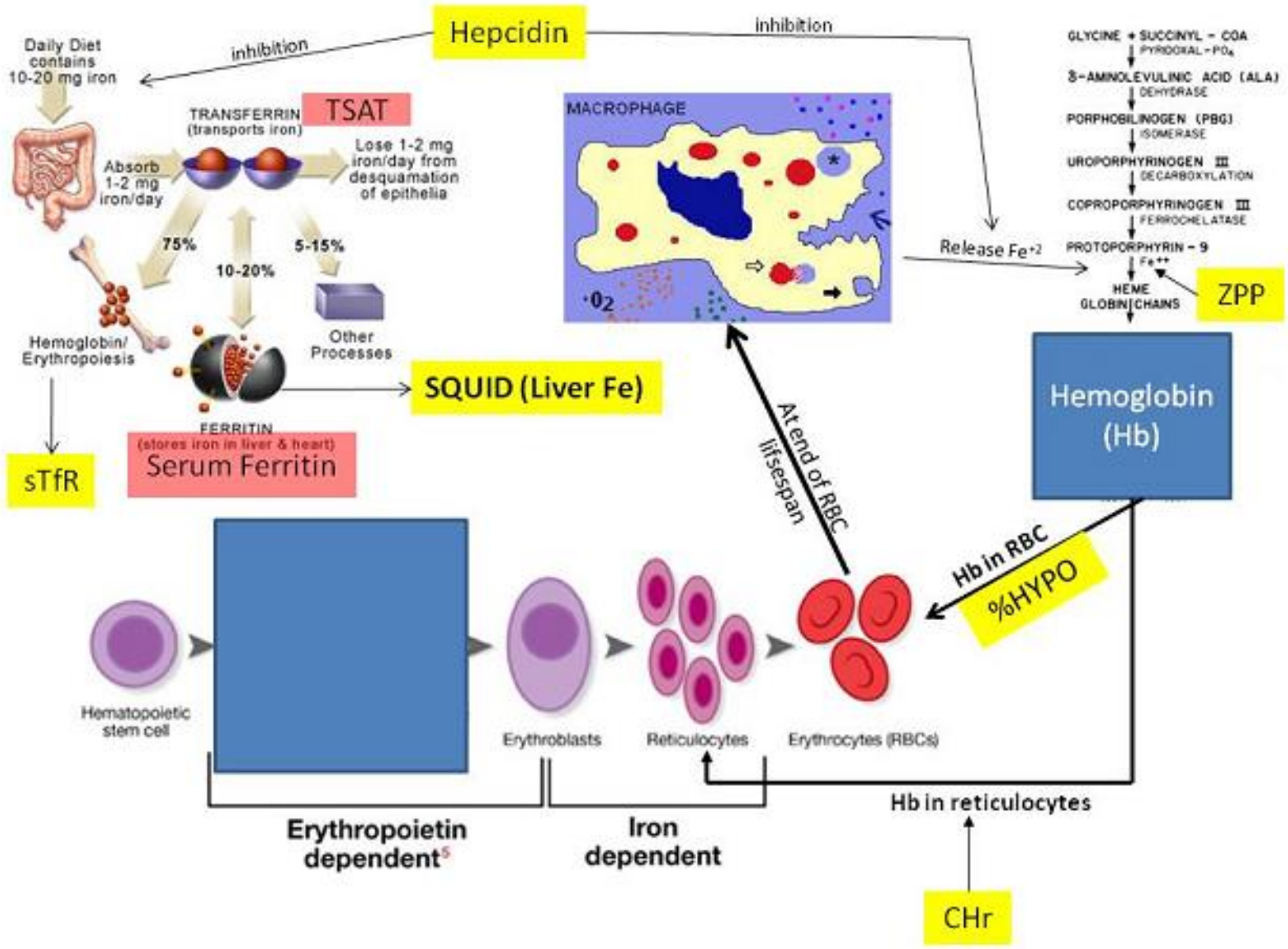
IDA


Hepcidin
inhibition
inhibition
Daily Diet contains 10-20 mg iron
TRANSFERRIN (transports iron)
TSAT
Absorb 1-2 mg iron/day
Lose 1-2 mg iron/day from desquamation of epithelia
75%
10-20%
5-15%
Hemoglobin/ Erythropoiesis
Other Processes
FERRITIN (stores iron in liver & heart)
Serum Ferritin
SQUID (Liver Fe)
sTfR
MACROPHAGE
Release Fe+2
GLYCINE + SUCCINYL - COA
δ-AMINOLEVULINIC ACID (ALA)
PORPHOBILINOGEN (PBG)
UROPORPHYRINOGEN III
COPROPORPHYRINOGEN III
PROTOPORPHYRIN - 9
HEME
GLOBIN CHAINS
ZPP
Hemoglobin (Hb)
At end of RBC lifesepan
Hb in RBC
%HYPO
Hematopoietic stem cell
Erythroblasts
Reticulocytes
Erythrocytes (RBCs)
Hb in reticulocytes
Erythropoietin dependent[5]
Iron dependent
CHr

IDA

FERRITIN CUTOFFS

RULE IN **<15 µg/L**

50-100 µg/L: possible IDA

RULE OUT **>100 µg/L** in assenza di infiammazione

(CRP assay !!)

Ferraro S. Clin Chem Lab Med 2012;50:1911

IDA: available biomarkers

	Advantages	Limitations
Screening		
1. Haemoglobin	Inexpensive	Low sensitivity
	Universally available	Low specificity
2. Transferrin saturation	Inexpensive	Wide diurnal variation
	Well established	Low specificity
3. Mean corpuscular haemoglobin	Widely available	Late indicator
	Well established	Low specificity
4. Zinc protoporphyrin	Portable assay	Automation difficult
	Inexpensive	Affected by lead exposure
5. Reticulocyte haemoglobin (CHr)	Early indicator	Limited availability
	Stability	Limited experience
Definitive		
1. Serum ferritin	Quantitative (iron stores)	Affected by inflammation
	Well standardized	Affected by liver disease
2. Serum transferrin receptor	Quantitative (tissue deficiency)	Lacks standardization
	Unaffected by inflammation	Affected by rHuEPO treatment
3. Bone-marrow iron	Well established	Invasive, expensive
	High specificity	Prone to error

Cook JD. Best Pract Clin Haematol 2005;18:319

Hepcidin, the essentials

•hepcidin is synthesized in the liver as a 84 amino acid pre-propeptide

•in addition to prohepcidin and hepcidin-25, 22, and 20 carboxy terminal amino acid forms of hepcidin are found in the circulation and/or urine

•hepcidin-25 is the bioactive form of hepcidin, the other forms have little or no biological activity.

•in humans it is part of the innate immune defence to remove iron sources for bacterial growing

•it is the main circulating inhibitor of iron flux from enterocytes, hepatocytes, macrophages and placental cells into the blood circulation

•these effects in part by binding to ferroportin, the main cellular iron-exporter in mammals

•large interindividual variation in concentration: wide reference intervals with limited use in interpretation

•hepcidin probably to be interpreted together with other indices of iron metabolism

•apparent circadian rhythm (increase during the day), no differences between sexes; lower in premenopausal women

Review

EASL JOURNAL OF HEPATOLOGY

Hepcidin in human iron disorders: Therapeutic implications☆

Antonello Pietrangelo

Centre for Hemochromatosis, Center for Advanced Research in Hepatology "Mario Coppo", University Hospital of Modena, Modena, Via del Pozzo 71, 41100 Modena, Italy

Journal of Hepatology **2011** vol. 54 | 173–181

MALDI-TOF MS

Key points 1. Essentials on hepcidin

- Structure: 84 amino acid prepropeptide containing N-terminal 24 amino acid endoplasmic reticulum targeting signal sequence, and a 35 amino acid proregion with a consensus furin cleavage site followed by the C-terminal 25 amino acid bioactive iron-regulatory hormone
- Metabolism:
  - Synthesis: mainly in the hepatocytes
  - Serum concentration: 20-200 ng/ml
  - Excretion: kidney
- Regulation:
  - Stimulators: high serum/hepatic iron, inflammatory cytokines, bone morphogenetic proteins (BMPs), ER stress
  - Inhibitors: low serum/hepatic iron, anemia-hypoxia, bone-marrow derived factors (GDF15, TWSG1), erythropoietin
- Activity: N-terminus interacts with the iron-exporter ferroportin in macrophages, enterocytes, hepatocytes and placental cells and causes its internalization and degradation, leading to decreased cell iron efflux

Hepcidin, the laboratory

- Hep tendency to aggregate and to stick to plastic material
- **Small** and compact structure which leaves scarce free antigenic structure and **high degree of hep conservation among many species**→ difficulties in making anti-hep specific antibodies
- **3** circulating hep isoforms (-25, -22 and -20), only one is active **(-25)** – the relevance of measuring only -25hep **not** systematically studied
- "**Round Robin 1**" (2009): good methods correlation, similar between-sample and analytical variations, but **very different hep concentrations** → **no** reference method, **no** validated commutable calibrator **or** other material for assay harmonization
- **Incomplete** and **hard comparison** of the the analytical characteristics of published assays for publication bias, no accordance to the STARD initiative
- **No** universal reference intervals and cut-off values for clinical reasoning

Table 1. Currently available hepcidin methods.

MS	Reference	Immunochemical	Reference	Hepcidin binding	Reference
HPLC/UPLC[a] MS/MS	(87;88)	c-ELISA	(13;29;98;99) (Bachem)	Q-TOF LC-MS	(92)
LC-MS/MS	(85;86;89)	c-RIA	(96;97) (Bachem)	HBD assay	(43)
MALDI-TOF MS	(13;30;91)	Sandwich ELISA	(100)		
SELDI-TOF MS	(90;94;95)				

[a] UPLC, ultra-high pressure LC; MS/MS, tandem MS; Q, quadrupole; HBD, hepcidin-binding domain.

Hepcidin, the laboratory

TABLE I. Characteristics of Methods Used for Plasma Hepcidin Measurements

Platform	Method	Hepcidin extraction		Standard	Purchased from	Reference
MS	HPLC-MS/MS	Reversed Phase	Internal	Synthetic Hepcidin-25 [13C6,15N1]Ile6, [13C6,15N1]Ile8	Bachem, in house made	[10]
MS	LC-MS/MS	WCX	Internal	Mouse synthetic Hepcidin 1	Peptide Int.	–
MS	LC-MS/MS	WCX magnetic nanoparticles	Internal	Synthetic Hepcidin-25 [13C6]Phe9, [15N,13C2]Gly20	Peptide Int., in house made	[11]
MS	LC-MS/MS	Reversed Phase	Internal	Synthetic Hepcidin-25 [13C9,15N1]Phe4	In house made	[14]
MS	LC-MS/MS	Reversed Phase	Internal	Synthetic Hepcidin-25 [13C8,15N3]	Peptide Int.	[9]
MS	LC-MS/MS	Protein ppt & SPE	Internal	Calcitonin gene-related peptide	Peptide Int.	[8]
MS	MALDI-TOF MS	None	Internal	Synthetic Hepcidin-25 [15N4,13C6]Arg16	Sigma-Genosys	[17]
MS	MALDI-TOF MS	WCX	Internal	Synthetic Hepcidin-24	Peptide Int.	[13,19]
MS	Q-TOF LC/MS	HBD and ACNppt	External	Synthetic Hepcidin-24[15N2,13C6]Lys24	Sigma AQUA	[18]
MS	SELDI-TOF MS	IMAC	External	Synthetic Hepcidin-25	Peptide Int.	[15]
MS	SELDI-TOF MS	IMAC	Internal	Synthetic Hepcidin-25[15N1,13C9]Phe9	AltaBioscience	[12]
IC	Competitive ELISA	None	External	Synthetic Hepcidin-25	Peptide Int.	[19]
IC	Competitive ELISA	None	External	Synthetic Hepcidin-25	Bachem	[20]
IC	Competitive ELISA	None	External	Synthetic Hepcidin-25	Bachem	*
IC	Competitive ELISA	None	External	Modified Hepcidin-25 fragment	DRG	[28]
IC	Competitive ELISA	None	External	Recombinant Hepcidin-25	In house made	[22]
IC	Competitive RIA	None	External	Synthetic Hepcidin-25	Bachem	[23]
IC	Competitive RIA	None	External	Synthetic Hepcidin-25	Peptide Int.	[24]
IC	Sandwich ELISA	None	External	Synthetic Hepcidin-25	Peptide Int.	[25]
IC	Sandwich ELISA	None	External	Recombinant Hepcidin-25	In house made	–
IC	HBD Method	None	Internal	^{125}I-Hepcidin-25	In house made	[21]

TABLE IV. The Algorithm to Calculate the Estimated Hepcidin Value (in nmol/L) from the HEPCON1 Value (nmol/L), Using a Linear Regression Model on the Native Samples Only

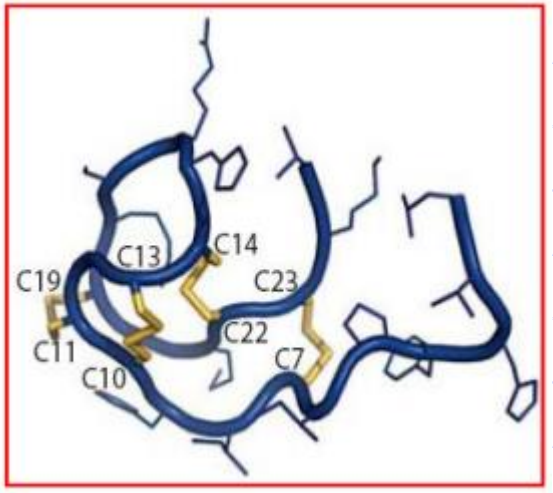
Method								SEM	R-square
IC1	Y	=	−2.67	+	0.79	×	HEPCON1	0.39	0.98
IC2	Y	=	−1.23	+	0.64	×	HEPCON1	0.45	0.96
IC3	Y	=	−0.11	+	0.38	×	HEPCON1	0.19	0.98
IC4	Y	=	−0.15	+	0.89	×	HEPCON1	0.54	0.97
IC5**	Y	=	1.75	+	1.29	×	HEPCON1	1.85	0.85
IC6	Y	=	11.37	+	2.42	×	HEPCON1	2.94	0.89
MS1	Y	=	0.62	+	0.29	×	HEPCON1	0.16	0.97
MS2	Y	=	0.87	+	0.44	×	HEPCON1	0.35	0.95
MS3	Y	=	−1.17	+	0.91	×	HEPCON1	0.44	0.98
MS4	Y	=	2.41	+	1.13	×	HEPCON1	0.46	0.99
MS5	Y	=	−2.92	+	0.74	×	HEPCON1	0.52	0.96
MS6	Y	=	1.19	+	0.94	×	HEPCON1	0.45	0.98
MS7**	Y	=	16.43	+	1.17	×	HEPCON1	2.66	0.69
MS8	Y	=	−1.19	+	1.06	×	HEPCON1	0.45	0.98
MS9	Y	=	1.25	+	0.50	×	HEPCON1	0.60	0.89
MS10*	Y	=	−0.36	+	0.38	×	HEPCON1	0.84	0.70

Am. J. Hematol. 87:977–983, 2012.

- "**Round Robin 2**" (2012): good methods correlation, similar between-sample and analytical variations, but **very different hep concentrations** → **no** reference method, **no** validated commutable calibrator **or** other material for assay harmonization
- **No** universal reference intervals and cut-off values for clinical reasoning

EPCIDINA

Problematiche metodologiche della sua determinazione



1. scarsa immunogenicità per limiti di conformazione molecolare.
2. alto grado di omologia tra i vertebrati
3. deriva da un propeptide (con peptide segnale)
4. tre isoforme presenti in circolo (Hepc-20, Hepc-22, Hepc-25) e non tutte biologicamente attive
5. tende ad aggregarsi, a legarsi alle proteine e ad appiccicarsi alla parete delle provette: precauzioni di prelievo!
6. **ritmo circadiano**
7. **elevata imprecisione dei saggi (specie su urine)**

METODO CONSIGLIATO: MALDI-TOF MS

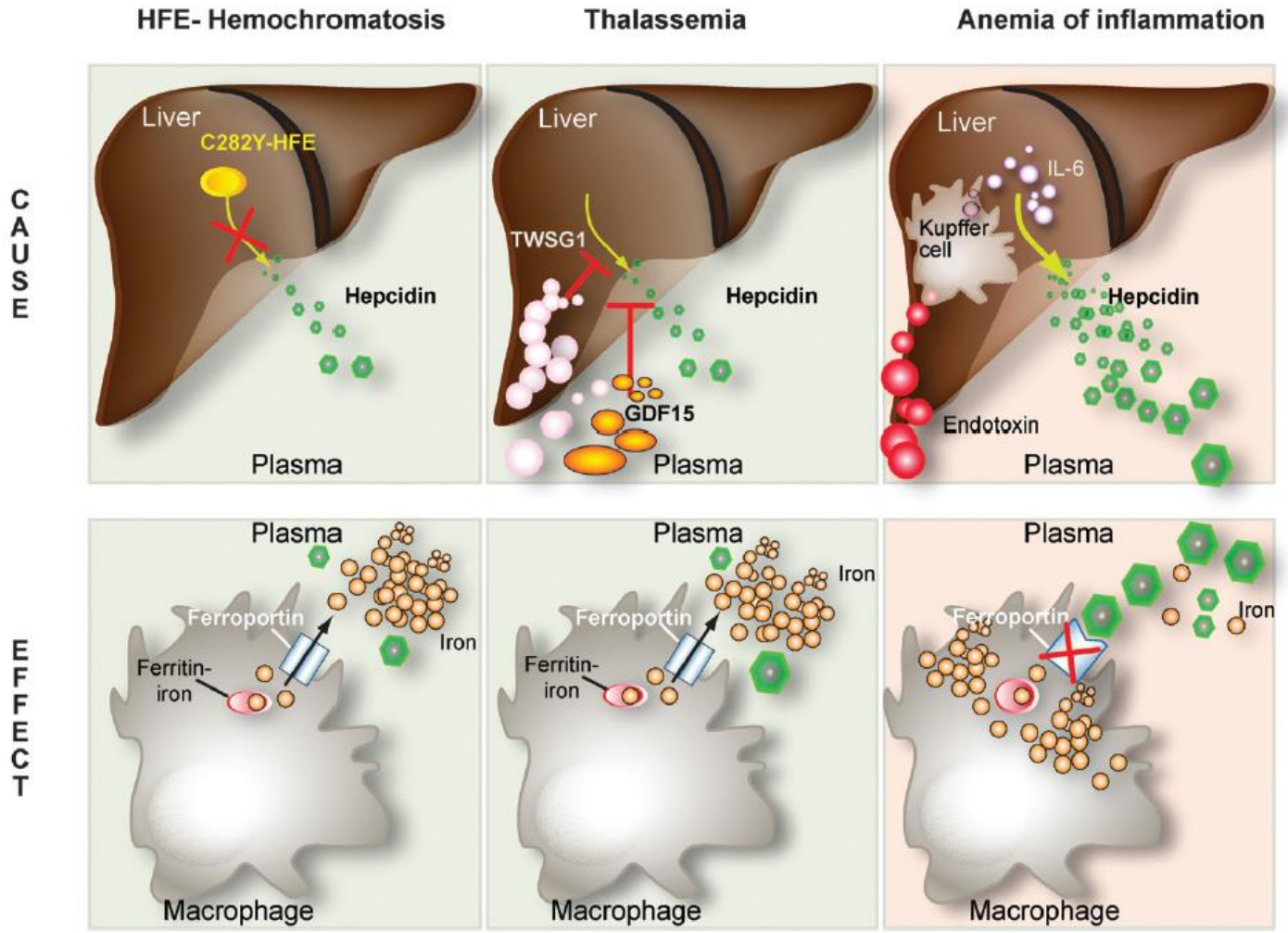


Fig. 2. Hepcidin in the pathogenesis of human hereditary and acquired iron disorders. Abnormal hepcidin regulation is responsible for iron disturbances in paradigmatic human disorders characterized by hepcidin deficiency (HFE-hemochromatosis and thalassemia) or hepcidin excess (anemia of chronic disease or anemia of inflammation). The Figure depicts the liver, as the main site of hepcidin production, and the macrophage, a site for hepcidin activity highly expressing ferroportin, the hepcidin-target. HFE-Hemochromatosis: In HFE-HC, functional loss of HFE due to the C282Y mutation, leads to impaired BMP/SMAD signaling and hepcidin attenuated transcriptional activity (see text for details). The effect of inadequate levels of circulating hepcidin leads to unchecked iron-export by ferroportin in the macrophage, responsible for progressive plasma iron load, tissue iron accumulation and disease. Thalassemia: In hereditary anemias associated with inefficient erythropoiesis, such as thalassemia at early pre-transfusion stages, erythroid factors induced by inefficient erythropoiesis (such as GDF15 and TWSG1) inhibits hepcidin transcription, and cause, in analogy with HFE-HC, hepcidin deficiency and unrestricted iron release from macrophages toward the bloodstream. Anemia of inflammation: During inflammatory states or infection, pathogen by-products may lead to cytokine overproduction, particularly from macrophages and resident Kupffer cells in the liver. The latter may release cytokines, such as IL-6, that stimulate hepcidin production by the hepatocytes. Prolonged hyper-hepcidinemia may then lead to iron sequestration in macrophages, hypoferremia and iron-restricted anemia. In spite of low serum iron, tissue iron excess and inflammatory mediators lead to elevation of serum ferritin.

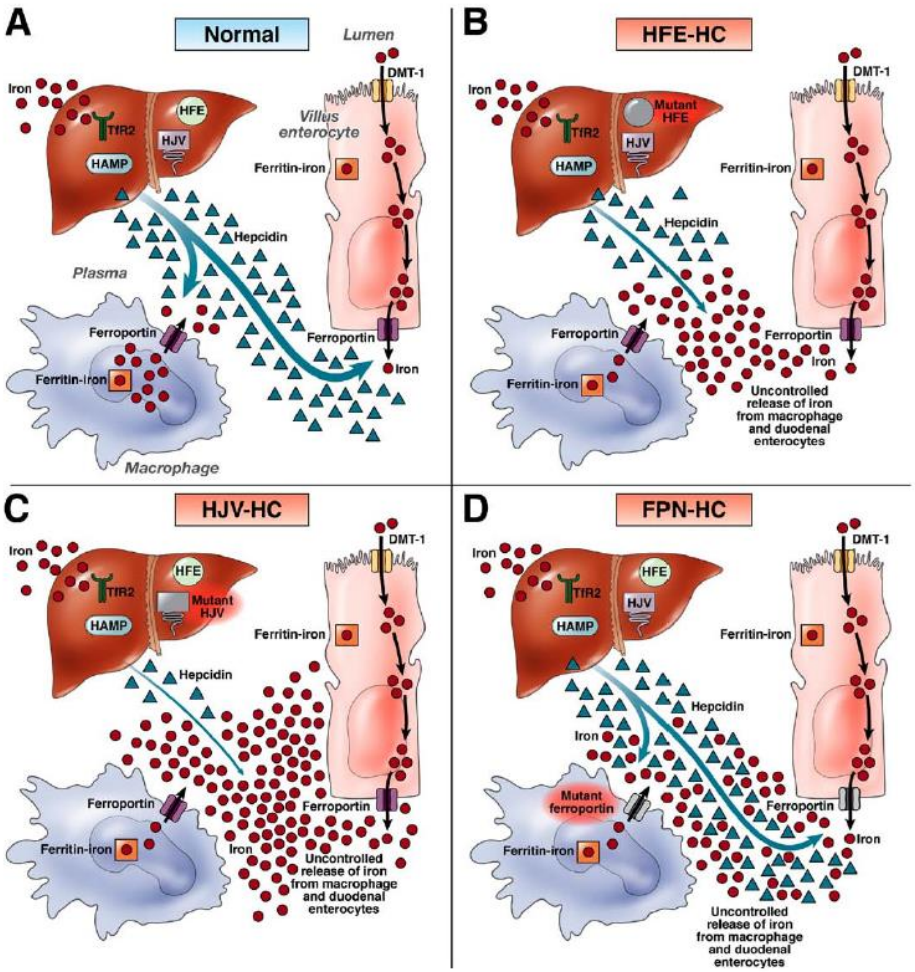


Figure 3. Hepcidin deficiencies are the central pathogenic factor in hemochromatosis. (*A*) In healthy subjects, hepcidin secreted by the liver regulates iron release from macrophages and duodenal enterocytes by interacting with the FPN expressed on their surfaces. HFE, TfR2, and HJV are all required to adjust hepcidin expression to current iron needs. Loss of any one of these hepcidin regulators leads to unrestricted flow of iron into the plasma iron pool. Some of the iron will be taken up by the bone marrow for erythropoiesis or by skeletal muscle for incorporation in myoglobin; some will be stored in the hepatocytes in the form of ferritin. The rest remains in circulation because, other than menstruation, the body has no effective way of eliminating iron. From the blood, the excess iron spills over into parenchymal tissues, where it causes oxidative damage to key cell structures. The characteristics of the circulatory iron loading depend on which hepcidin regulator is lost. The buildup can be (*B*) mild-moderate and gradual in the absence of HFE or (*C*) massive and rapid, as it is when HJV is lost, and this results in milder (*HFE* associated) or more severe forms of hemochromatosis (*HJV* or *HAMP* associated). Loss of TfR2 probably causes an intermediate level of hepcidin deficiency that produces an adult-onset iron-loading syndrome that appears somewhat earlier than HFE-related hemochromatosis and is somewhat more severe. (*D*) Classic hemochromatosis can also be associated with rare mutations in FPN that render the iron exporter unresponsive to hepcidin. Although hepcidin is appropriately synthesized and released in response to rising plasma levels of iron, the mutant FPN continues to release dietary iron into the plasma. Modified with permission from Pietrangelo A. Hereditary hemochromatosis—a new look at an old disease. N Engl J Med 2004;350:2383–2397.

Clinical Chemistry 57:12
000–000 (2011)

Hepcidin in Human Iron Disorders: Diagnostic Implications

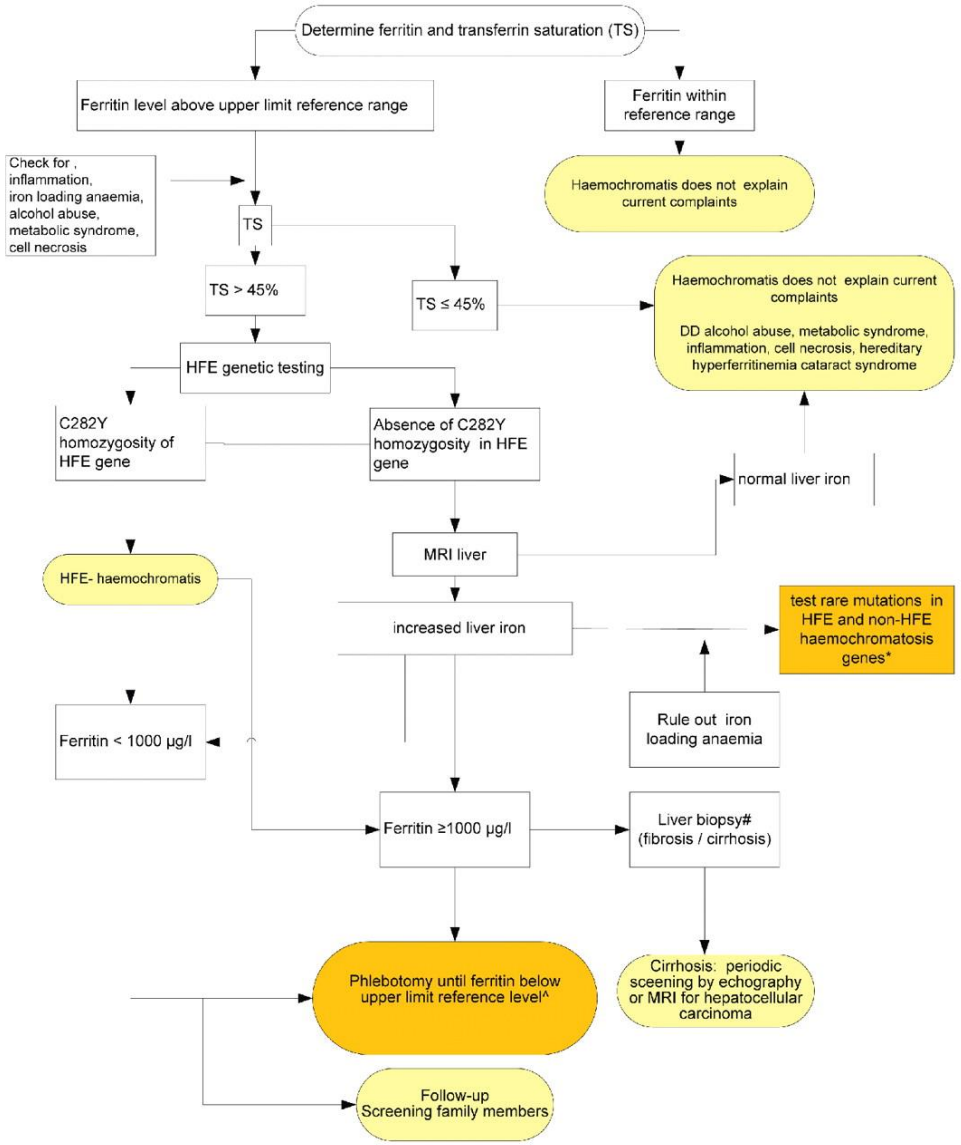
Joyce J.C. Kroot,[1] Harold Tjalsma,[1,2] Robert E. Fleming,[3] and Dorine W. Swinkels[1,2*]

Table 2. Most promising applications of serum hepcidin in diagnostic medicine.

Disease condition	Expected hepcidin concentrations[a]	Potential added value of hepcidin diagnostics
Classical hereditary hemochromatosis	Low, maybe compensated by iron overload over time	Screen for the presence of hemochromatosis
		Predict which homozygotes will be at risk for iron overload
		Determine the phlebotomy interval
		Prioritize genes to be investigated
Iron-loading anemias	Low, maybe compensated by transfusional iron overload over time	Identify the most severely affected patients
		Predict and monitor (parenchymal) iron overload
Acquired forms of iron overload	Miscellaneous	A marker of iron dysregulation
Iron-refractory iron deficiency anemia	Inappropriately high	Screen for primary defect in hepcidin regulation
Inflammation and infection	High	Differentiate ACD and IDA
		Guide iron supplementation therapy
Chronic kidney diseases	High, decreases upon EPO treatment	Predict EPO response
		Guide treatment with EPO, intravenous iron
Acute kidney injury	Low (urine)	Diagnose acute kidney injury
Treatment with hepcidin antagonists and agonists	Depends on the disorder	Monitor and assess indications

[a] In serum, unless stated otherwise.
ACD, anemia of chronic disease; IDA, iron deficiency anemia; EPO, erythropoietin.
*, ●●●.

Proposed diagnostic and therapeutic algorithm for patients with suspected hemochromatosis.



Swinkels D W , Fleming R E Haematologica 2011;96:485-488

haematologica
the hematology journal

Iron refractory iron deficiency anemia (IRIDA)

- Anemia ereditaria recessiva
- Difetto nel gene TMPRSS6 codificante per Matriptasi-2, proteina transmembrana ad attività proteasica essenziale nella down-regolazione di hep
- Anemia microcitica e ipocromica a precoce insorgenza
- Bassa saturazione Trf
- Hep inappropriatamente alta/normale, in contrasto con il ridotto stato Fe
- NO risposta Fe per os, Tx con Fe IV→ manipolazione della via regolatoria di hep

Iron refractory iron deficiency anemia

Luigia De Falco,[1,*] Mayka Sanchez,[2,3,*] Laura Silvestri,[4] Caroline Kannengiesser,[5,6] Martina U. Muckenthaler,[7,8] Achille Iolascon,[1,9] Laurent Gouya,[10,11] Clara Camaschella,[4] and Carole Beaumont[6,10,12]

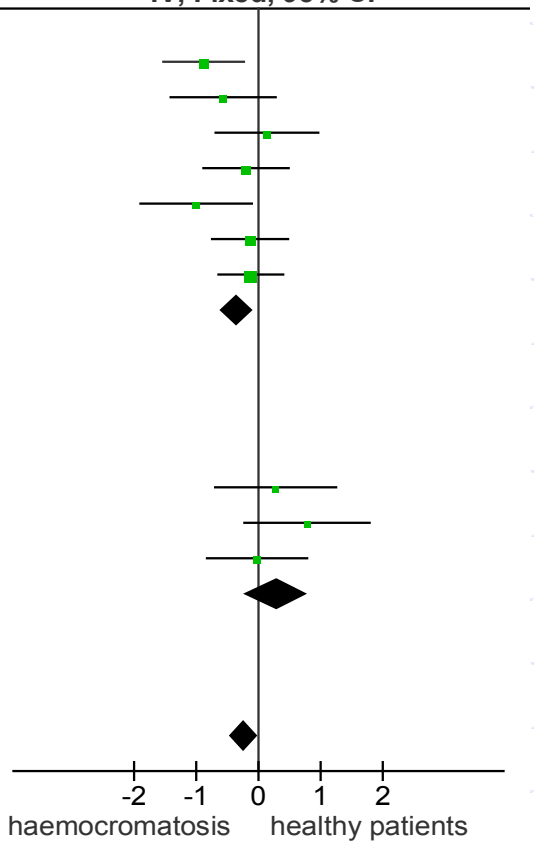
haematologica | 2013; 98(6)

Hepcidin

- SR for hepcidin measurement for **HH diagnosis**; comparison of the serum and urine hepcidin concentration in healthy controls and in HH HFE patients (C282Y/C282Y homozygotes and C282Y/H63D compound heterozygotes)
- Medline and Embase research produced 414 records
  - 43 papers chosen on the basis of the abstracts
  - 35 papers **not** included (studies about method accuracy, methods comparison and about mRNA expression)
  - 8 papers included
- **Serum** (6 papers) and **urine** (3 papers) hepcidin measurements (Elisa and MS)

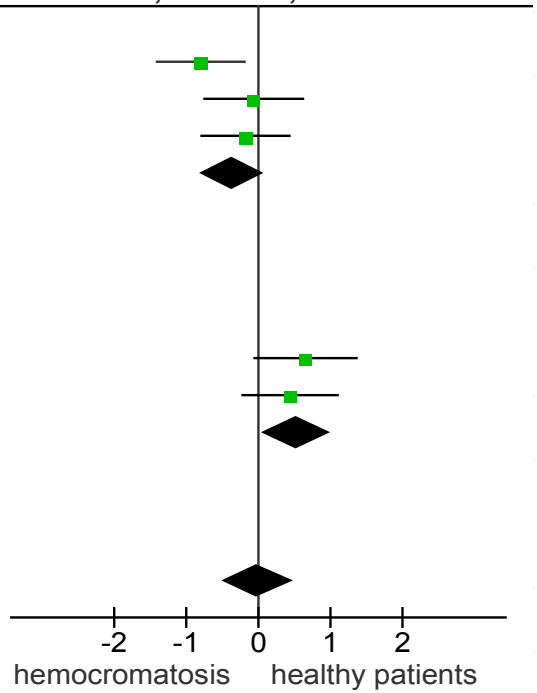
- **Many limits**:
  - Too less papers
  - Only observational studies
  - Alwais not good quality
  - Different analytical methods and unity of measure
  - Big reporting bias → data shown without the appropriate measure of dispersion; only p value reported

Study or Subgroup	Experimental Mean	SD	Total	Control Mean	SD	Total	Weight	Std. Mean Difference IV, Fixed, 95% CI
2.1.1 C282Y/C282Y								
Floreani 2011	3.2	1.9	10	13.7	12.2	150	12.8%	-0.88 [-1.53, -0.23]
Girelli 2011	41.9	38.23	10	59.6	22.06	13	7.6%	-0.57 [-1.41, 0.28]
Girelli 2011	12.2	12.36	10	10.3	14.13	13	7.9%	0.14 [-0.69, 0.96]
Kemna 2007	1.6	14.85	14	4.38	12.7	20	11.5%	-0.20 [-0.88, 0.49]
Sangwaiya 2011	13.1	16.5	9	32.9	20.5	14	6.7%	-1.00 [-1.90, -0.10]
Schwarz 2011	14.2	20	14	21.5	61.2	39	14.4%	-0.13 [-0.74, 0.48]
vanDijk 2008	5.2	5.8	22	7.6	23.8	40	19.8%	-0.12 [-0.64, 0.40]
Subtotal (95% CI)			**89**			**289**	**80.7%**	**-0.35 [-0.60, -0.09]**
Heterogeneity: Chi² = 7.61, df = 6 (P = 0.27); I² = 21%								
Test for overall effect: Z = 2.62 (P = 0.009)								
2.1.2 C282/H63D								
Girelli 2011	65.1	7.5	6	59.6	22.06	13	5.7%	0.28 [-0.70, 1.25]
Girelli 2011	22.4	16.24	6	10.3	14.13	13	5.3%	0.78 [-0.22, 1.79]
Schwarz 2011	20.2	26.6	7	21.5	61.2	39	8.3%	-0.02 [-0.83, 0.78]
Subtotal (95% CI)			**19**			**65**	**19.3%**	**0.29 [-0.24, 0.81]**
Heterogeneity: Chi² = 1.50, df = 2 (P = 0.47); I² = 0%								
Test for overall effect: Z = 1.07 (P = 0.29)								
Total (95% CI)			**108**			**354**	**100.0%**	**-0.22 [-0.46, 0.01]**
Heterogeneity: Chi² = 13.55, df = 9 (P = 0.14); I² = 34%								
Test for overall effect: Z = 1.89 (P = 0.06)								
Test for subgroup differences: Chi² = 4.45, df = 1 (P = 0.04), I² = 77.5%								



Serum hepcidin - at diagnosis

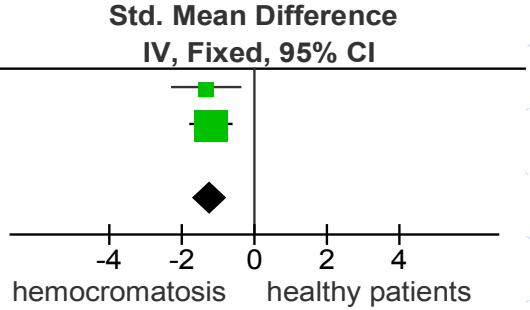
Study or Subgroup	Experimental Mean	SD	Total	Control Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI
1.1.1 C282Y/C282Y								
Bozzini 2008	0.2	0.17	18	0.49	0.43	30	20.9%	-0.80 [-1.41, -0.19]
Kemna 2007	0.66	7.3	14	1.45	14.53	20	19.4%	-0.06 [-0.75, 0.62]
Piperno 2007	27.8	50.91	19	36.44	44.6	23	20.9%	-0.18 [-0.79, 0.43]
Subtotal (95% CI)			**51**			**73**	**61.2%**	**-0.36 [-0.81, 0.09]**
Heterogeneity: Tau² = 0.06; Chi² = 3.07, df = 2 (P = 0.22); I² = 35%								
Test for overall effect: Z = 1.57 (P = 0.12)								
1.1.2 C282/H63D								
Bozzini 2008	0.79	0.5	11	0.49	0.43	30	18.9%	0.66 [-0.05, 1.36]
Piperno 2007	83.4	157.6	15	36.44	44.6	23	19.9%	0.44 [-0.22, 1.10]
Subtotal (95% CI)			**26**			**53**	**38.8%**	**0.54 [0.06, 1.02]**
Heterogeneity: Tau² = 0.00; Chi² = 0.19, df = 1 (P = 0.66); I² = 0%								
Test for overall effect: Z = 2.20 (P = 0.03)								
Total (95% CI)			**77**			**126**	**100.0%**	**-0.01 [-0.51, 0.50]**
Heterogeneity: Tau² = 0.22; Chi² = 11.97, df = 4 (P = 0.02); I² = 67%								
Test for overall effect: Z = 0.02 (P = 0.98)								
Test for subgroup differences: Chi² = 7.16, df = 1 (P = 0.007), I² = 86.0%								



Urin hepcidin - at diagnosis

Study or Subgroup	Experimental Mean	SD	Total	Control Mean	SD	Total	Weight	Std. Mean Difference IV, Fixed, 95% CI
Sangwaiya 2011	0.3	0.3	9	1.1	0.7	14	26.6%	-1.33 [-2.26, -0.39]
vanDijk 2008	1.8	4.1	22	10.3	8.17	40	73.4%	-1.20 [-1.76, -0.63]
Total (95% CI)			**31**			**54**	**100.0%**	**-1.23 [-1.71, -0.75]**

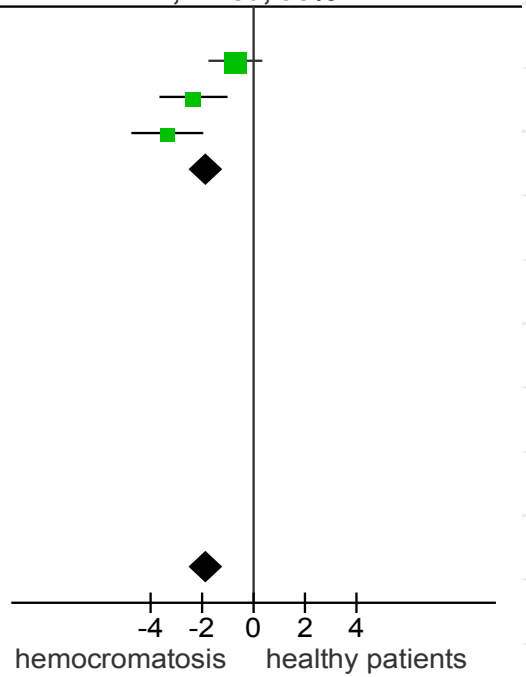
Heterogeneity: Chi² = 0.06, df = 1 (P = 0.81); I² = 0%
Test for overall effect: Z = 4.99 (P < 0.00001)



Hepcidin/ferritin ratio - at diagnosis/screening

C282Y/C282Y as patient group for both

Study or Subgroup	Experimental Mean	SD	Total	Control Mean	SD	Total	Weight	Std. Mean Difference IV, Fixed, 95% CI
2.2.1 C282Y/C282Y								
Girelli 2011	1.5	0.5	6	10.3	14.13	13	46.3%	-0.71 [-1.71, 0.29]
Girelli 2011	12.8	9	6	59.6	22.06	13	28.4%	-2.33 [-3.61, -1.06]
Sangwaiya 2011	18	10.7	9	75	19.08	14	25.3%	-3.35 [-4.70, -2.00]
Subtotal (95% CI)			**21**			**40**	**100.0%**	**-1.84 [-2.52, -1.16]**
Heterogeneity: Chi² = 10.28, df = 2 (P = 0.006); I² = 81%								
Test for overall effect: Z = 5.29 (P < 0.00001)								
2.2.2 C282/H63D								
Girelli 2011	1.5	4.3	3	10.3	14.13	13	0.0%	-0.63 [-1.91, 0.65]
Girelli 2011	7	23.67	3	59.6	22.06	13	0.0%	-2.23 [-3.77, -0.69]
Subtotal (95% CI)			**6**			**26**	**0.0%**	**-1.29 [-2.27, -0.30]**
Heterogeneity: Chi² = 2.45, df = 1 (P = 0.12); I² = 59%								
Test for overall effect: Z = 2.56 (P = 0.01)								
Total (95% CI)			**21**			**40**	**100.0%**	**-1.84 [-2.52, -1.16]**
Heterogeneity: Chi² = 10.28, df = 2 (P = 0.006); I² = 81%								
Test for overall effect: Z = 5.29 (P < 0.00001)								
Test for subgroup differences: Not applicable								



Serum hepcidin – HH iron depleted patients versus healthy controls

Table 1. Characteristics of patients with *HFE* hemachromatosis and controls at the time of testing.

	Cases (n=25)				Controls (n=13)
	At diagnosis (n=16)		After iron depletion (n=9)		
	C282Y/C282Y (n=10)	C282Y/H63D (n=6)	C282Y/C282Y (n=6)	C282Y/H63D (n=3)	
Age (years)	47±12	42±12	38±9	51±5	35±12*
Transferrin saturation (%)	70.5 (46-83)°	45 (37-57)	50.5 (46-58)	26 (23-48)	34 (30-41)
Serum ferritin (µg/L)	920±310	705±326	47.5±23.2	53.3±12.1	120±62^
Hemoglobin (g/dL)	14.7±0.7	15.8±1.1	14.8±0.7	15.3±0.2	15.1±0.9
Liver iron content‡ (µmol/g)	259±54	170±51	-	-	-
Iron removed (g)	-	-	4.2±2.9	3.1±1.1	-
Serum hepcidin (ELISA) (ng/mL)	41.9 (24-65.6)	65.1 (50.8-71.1)	12.8 (1-20)	7 (6.6-33.8)	59.6 (41.6-71.6)
Serum hepcidin (MS) (ng/mL)	12.2 (7.9-19.2)	22.4 (13.1-35.4)	1.5 (1.5-1.5)	1.5 (1.5-6.4)	10.3 (7-18)

*Data are presented as mean ± standard deviation or median with interquartile ranges (in brackets). *P=0.02,* versus *C282Y/C282Y at diagnosis. °P=0.04,* versus *C282Y/H63D at diagnosis. ^P=0.004* versus *all patients after iron depletion. ‡measured by magnetic resonance imaging (normal value < 36 µmol/g); ELISA: enzyme-linked immunosorbent assay; MS: mass spectrometry.*

Could Hepcidin have a role in monitoring phlebotomy and assessing the ferritin target and frequency of phlebotomy ?

Serum hepcidin – HH iron depleted patients versus healthy controls

Review

EASL JOURNAL OF HEPATOLOGY

Hepcidin in human iron disorders: Therapeutic implications☆

Antonello Pietrangelo

Journal of Hepatology 2011 vol. 54 | 173–181

Table 2. Hepcidin therapy in human iron disorders.

Disorder	Hepcidin levels	Known or postulated causes of hepcidin abnormality	Current therapy	Hepcidin therapy
Hereditary hemochromatosis	Low	Decreased hepcidin transcription due to genetic loss of proteins involved in the BMP/SMAD signaling	Phlebotomy (if contraindicated: iron chelators)	Agonist
Iron loading anemias (with inefficient erythropoiesis)	Low	Decreased hepcidin transcription due to anemia hypoxia/low iron/high EPO/bone marrow derived factors	None	Agonist
Anemia of chronic disease (rheumatologic disorders; IBD; CKD; multiple mielomas etc.)	High	Increased hepcidin transcription due to cytokines, bacterial by-products, ER stress	Iron supplements/ EPO/ESA	Antagonist
Hereditary iron-refractory iron deficiency anemia (IRIDA)	High	Increased hepcidin transcription due to genetic loss of TMPRSS6	I.V. iron	Antagonist
Hepcidin-producing hepatic adenomas	High	Increased hepcidin production by adenomas	Surgery	Antagonist

BMP, bone morphogenetic proteins; SMAD, Sma and mothers against decapentaplegic homolog; EPO, erythropoietin; ESA, erythropoiesis stimulating agents; TMPRSS6, transmembrane serine protease 6 gene; IBD, inflammatory bowel disease; CKD, chronic kidney diseases.

SATURAZIONE DELLA TRANSFERRINA: RUOLO DIAGNOSTICO

IRON OVERLOAD

Screening test for finding C282Y homozygotes in the general population (prevalence: **0.75%**)

2 CONSECUTIVE TEST

CUTOFF for TS

55%

50%

	MEN	WOMEN
SPECIFICITY %	99.6	99.4
SENSITIVITY %	90.0	55.0
PPV %	64.3	43.0
NPV %	99.9	99.7

Szőke D. Clin Chim Acta 2012;413:1184

SATURAZIONE DELLA TRANSFERRINA: RUOLO DIAGNOSTICO

IRON OVERLOAD

EASL raccomanda fortemente l'uso della **TS** (senza definirne il cut-off) come **esame di screening per** eventualmente eseguire **gli esami genetici** (gene HFE) per HH nei:

- **soggetti con sospetto IO:** eseguire TS (e ferritina) a digiuno → se elevata → analisi genetica delle mutazioni del gene HFE

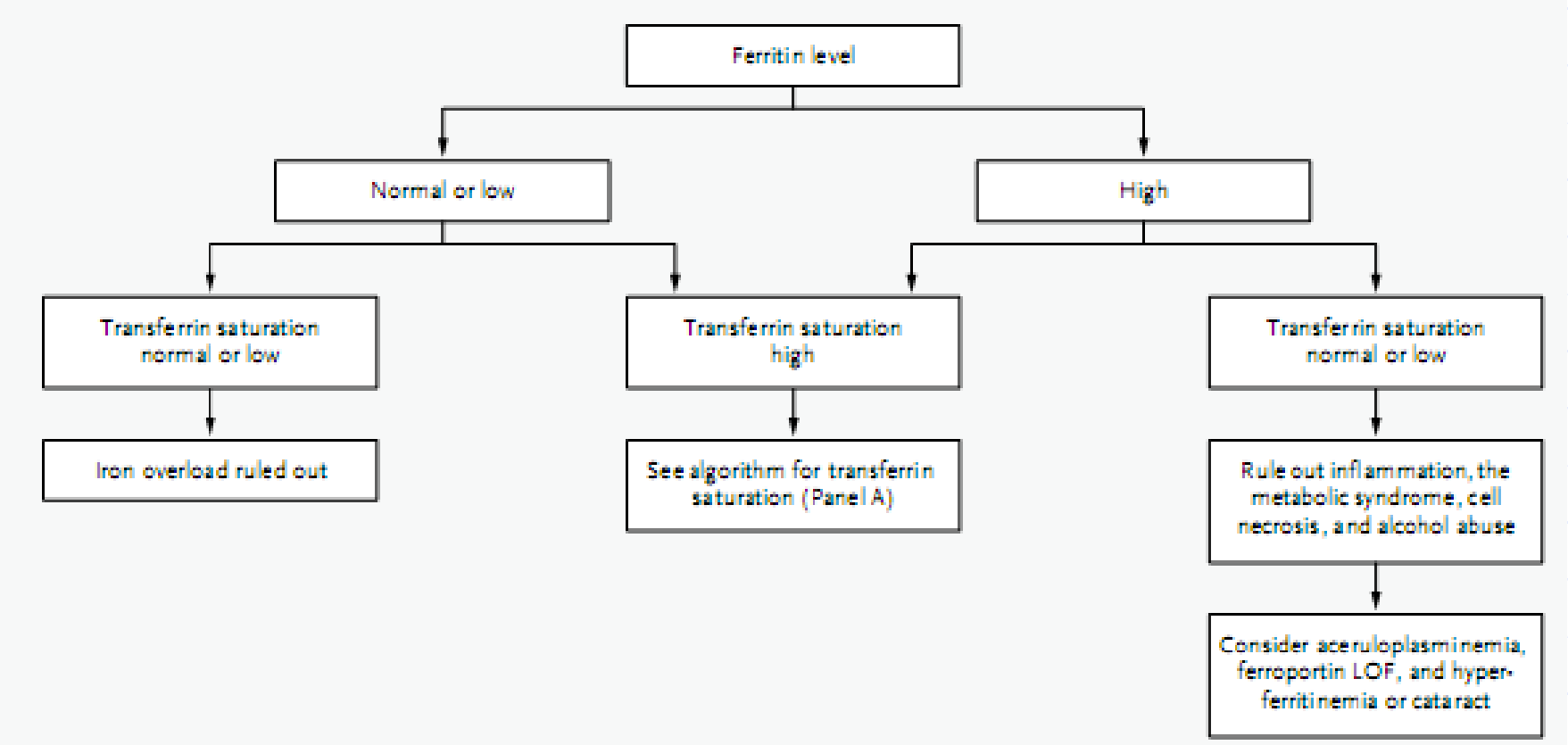
(evidenza di grado elevato)

-**pazienti ricoverati in reparti specialistici** (epatologia o gastroenterologia), eseguire TS (e ferritina) → se elevata → analisi genetica delle mutazioni del gene HFE

(evidenza di grado moderato)

Szőke D. Biochim Clin 2012;36:339

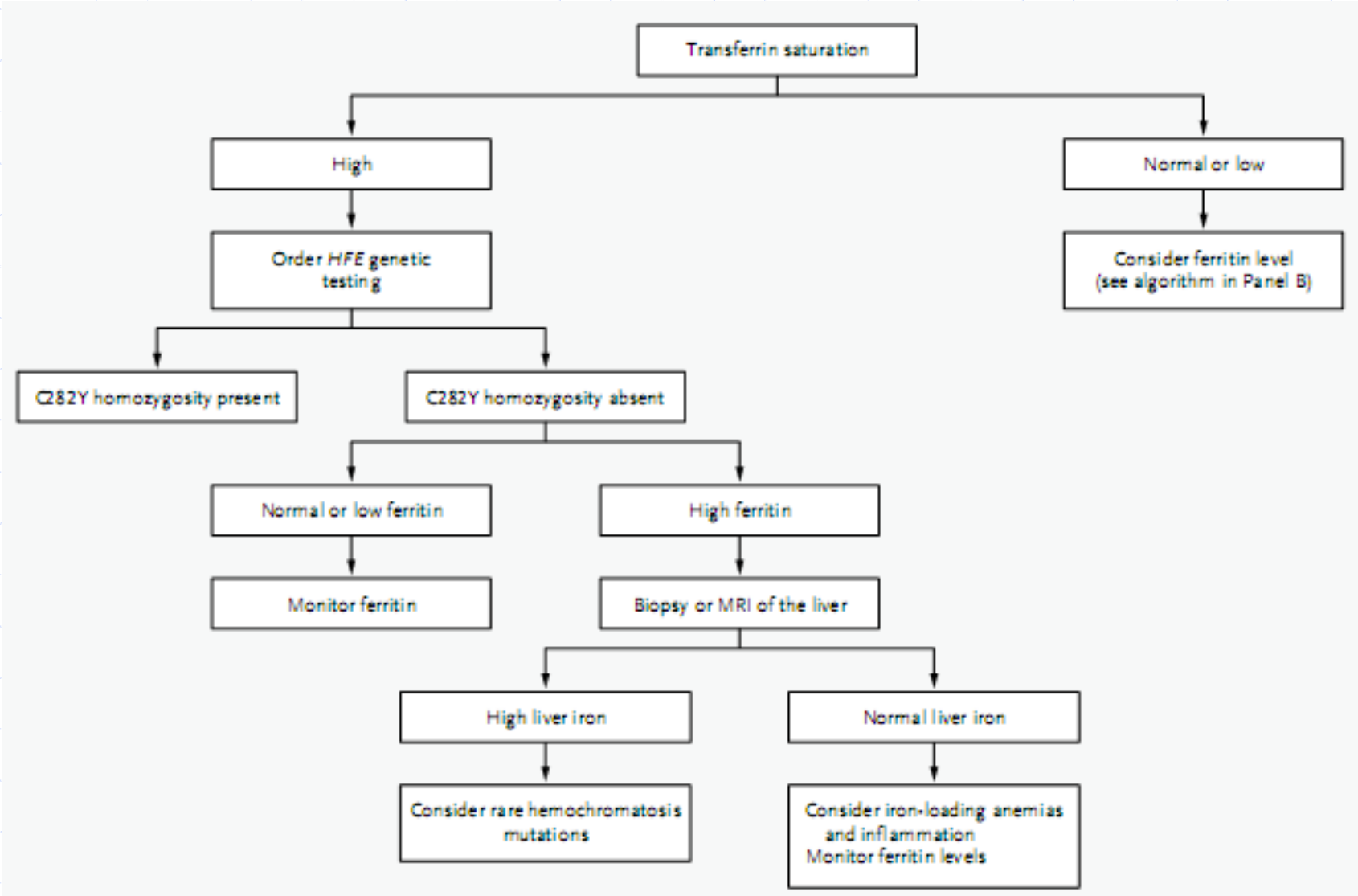
IRON OVERLOAD



F ferritina > 200ng/ml, TS > 45%
M ferritina > 300ng/ml, TS > 50%

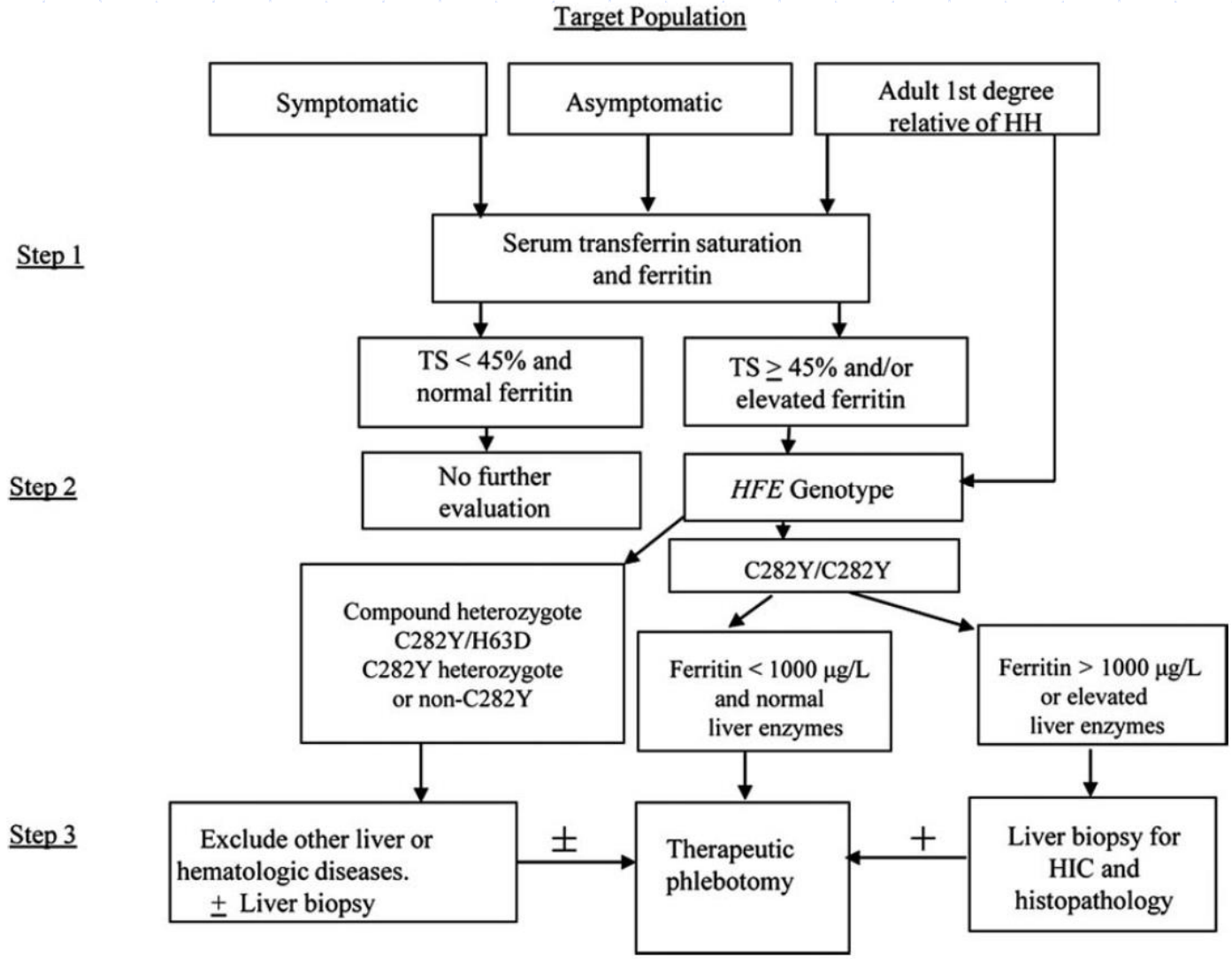
Fleming RE et al. NEJM 2012;366:348

IRON OVERLOAD



Fleming RE et al. NEJM 2012;366:348

IRON OVERLOAD



Hepatology, Vol 54, No1, 2011

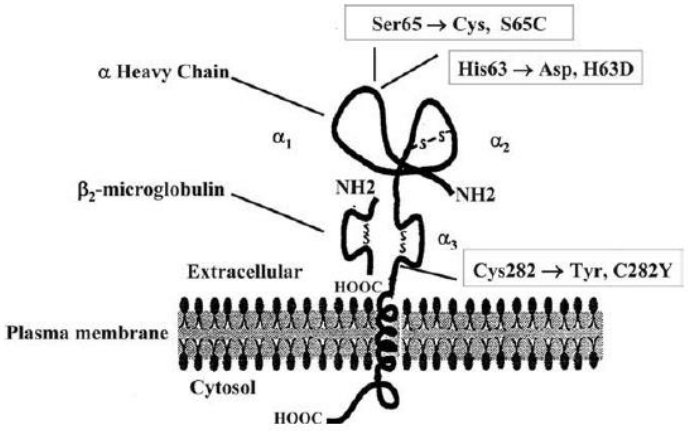
IRON OVERLOAD

Table 4. Prevalence of C282Y Homozygotes Without Iron Overload in Large Screening Studies

Population Sample	Country	*n*	Prevalence of Homozygotes	C282Y Homozygotes with Normal Ferritin Level (%)
Primary care ([12])	USA	41,038	1 in 270	35
General public ([11])	Norway	65,238	1 in 220	13
Primary care ([6])	North America	99,711	1 in 333	31
General public ([10])	Australia	29,676	1 in 146	32
Total		235,663	1 in 240	30

Table 5. Symptoms in Patients with HH

- Asymptomatic
  - Abnormal serum iron studies on routine screening chemistry panel
  - Evaluation of abnormal liver tests
  - Identified by family screening
- Nonspecific, systemic symptoms
  - Weakness
  - Fatigue
  - Lethargy
  - Apathy
  - Weight loss
- Specific, organ-related symptoms
  - Abdominal pain (hepatomegaly)
  - Arthralgias (arthritis)
  - Diabetes (pancreas)
  - Amenorrhea (cirrhosis)
  - Loss of libido, impotence (pituitary, cirrhosis)
  - Congestive heart failure (heart)
  - Arrhythmias (heart)



Hepatology, Vol 54, No1, 2011

PARAMETRI RBC e RET

- MCH, MCV, MCHC: sono gli indici tradizionali.

- MCH deriva da Hb e RBC, misure caratterizzate da notevoli precisione ed accuratezza dai moderni analizzatori
- MCHC deriva da Hb e HCT (RBC x MCV)
- MCV incompletamente standardizzato, sensibile allo storage, tempo dipendente
- MCV e MCH derivano dalla intera massa di RBC circolante e le loro modifiche sono lente a realizzarsi e, quindi, non hanno valore clinico per evidenziare l'acuta insorgenza di carenza marziale e precocemente la risposta ad ESA
- MCHC nonha uso clinico

- MCH e MCV alla diagnosi e per valutare il trend lungo settimane o mesi.

PARAMETRI RBC e RET

- RBC ipocromici ((IPO%, Hypo-He%, LHD (low haemoglobin density)).

  Originariamente refertati solo da Bayer/Siemens, gli RBC ipocromici sono definiti da una concentrazione di Hb < 28 g/dL.

  L'utilità clinica nella diagnosi di FID nei pazienti con CKD con EPO è stata descritta positivamente in numerosi studi a partire dal 1992.

  Un valore > 6% appare essere superiore a ferritina, sTfR, TS% nel differenziare IDA da FID/ACD in CKD

  Limiti legati alla preanalitica (parametro tempo dipendente)

  Gli altri parametri, generati da tecnologie Sysmex e Coulter, hanno teoricamente il medesimo significato. Pochi studi reperibili.

  LHD deriva dalla trasformazione matematica di MCHC, correla con IPO%

- IPO% è il parametro meglio descritto in diagnosi di FID e ha il il maggiore livello di evidenza

PARAMETRI RBC e RET

- CHr (MCH dei RET), originariamente descritto da Bayer/Siemens

  Attualmente fornito anche dalla tecnologia Sysmex mediante il parametro Ret-He (trasformazione logaritmica di Ret-Y).

  I due parametri indicano la stessa quantità, espressa in pg.

- Red blood cell size factor (Rsf), derivata dalla radice quadrata del prodotto di MCV x MCVr.

  Buona correlazione con CHr, SENS lievemente maggiore e medesima SPEC nella diagnosi IDA/FID; FID/ACD per valori > 87.7 fL, IDA se < IDA

- CHr < 29 pg predice IRE nei pazienti con carenza di ferro e nei pazienti in terapia con ESA (erythropoiesis-stimulating agent)
- Ret-He < 25 pg predice FID in terapia ESA

  Ret-He con cut-off 25 pg aiuta nella dx IDA/ACD

Analisi Reticolociti

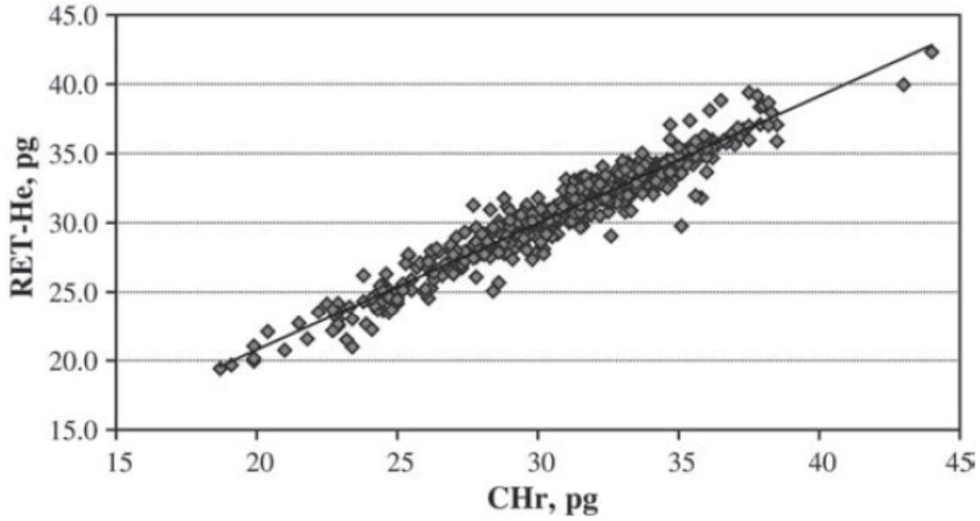


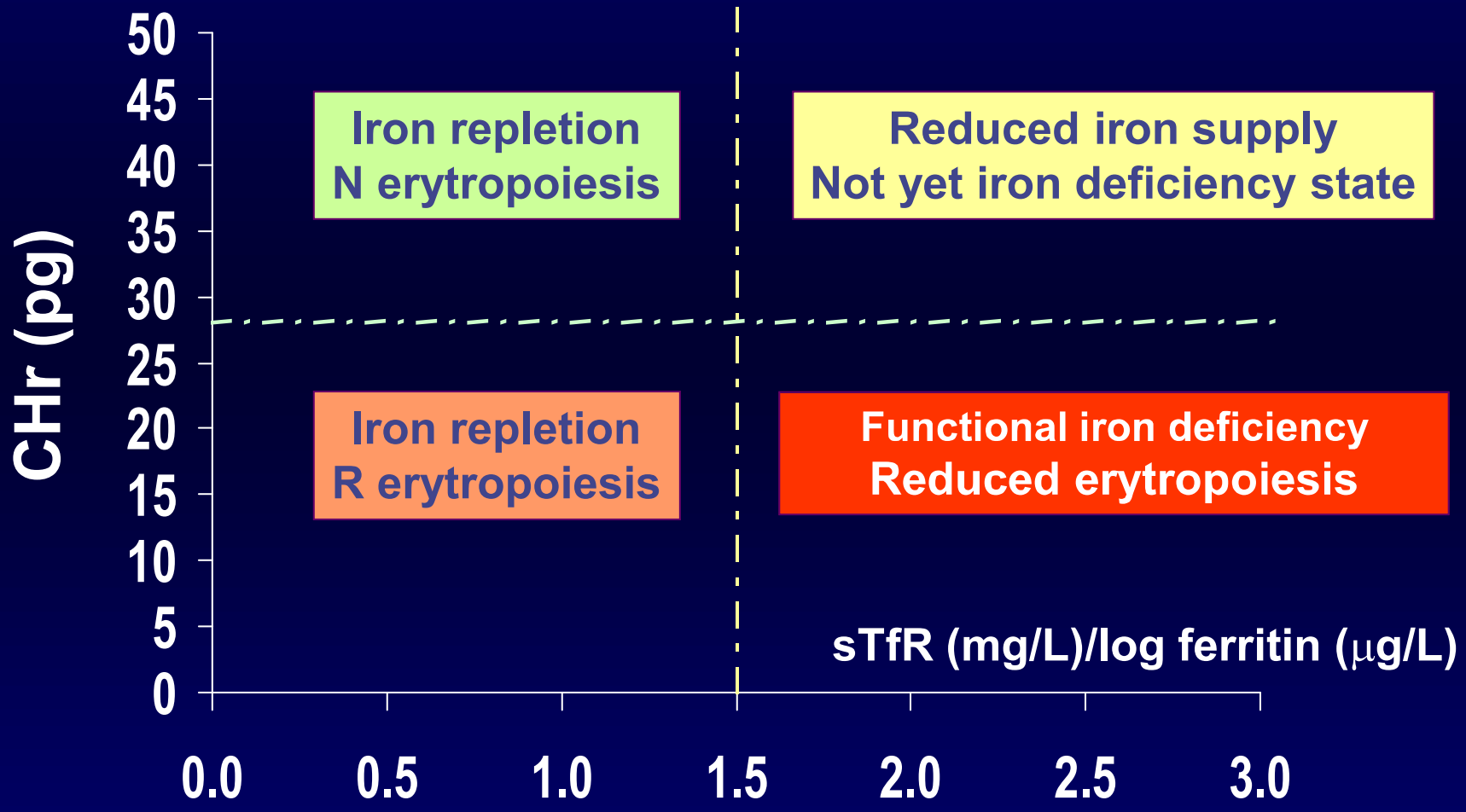
Figure 4 Transformation of RET-Y for 474 patients into hemoglobin equivalent units (pg) using the regression line obtained from the RBC-Y/MCH curve in Figure 2. Equation for the line: $y = 0.9182x + 2.42$ ($r^2 = 0.92$).

- Per CHr il range centrale 95% comprende 28-35 pg
- Dopo conversione di RET-Y in RET-He, il range 95% comprende a 28.2-35.7 pg

- Parametri con filosofia analitica differente, ma che misurano la stessa componente cellulare

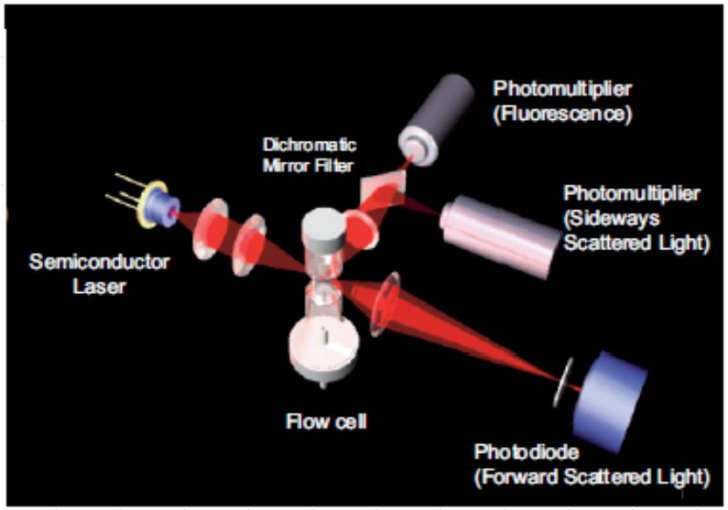
CCLM 2005;45:1193

Diagnostic plot for the identification of iron deficiency in anemic patients without acute-phase reaction

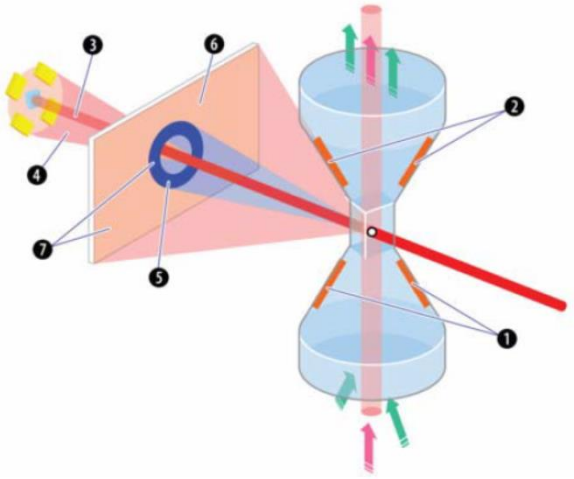


Thomas C. Clin Chem 2002;48:1066

Referto strumentale ematologico: conteggi, curve di distribuzione, citogrammi

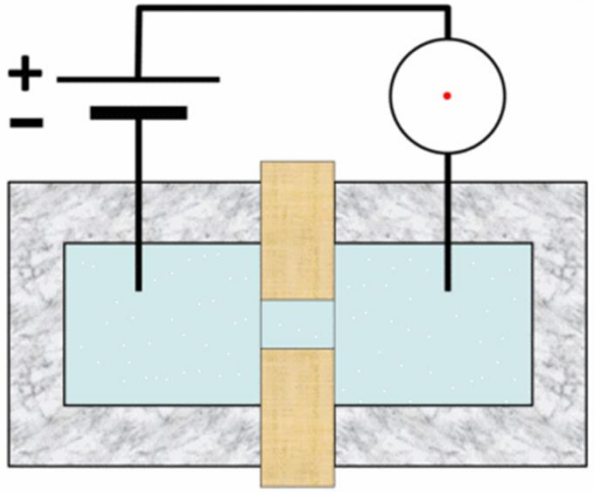


- I principi analitici fondamentali sono utilizzati nelle differenti tecnologie presenti sul mercato:
  - impedenza elettrica (metodo Colulter)
  - assorbimento e diffrazione della luce
  - fluorescenza
- La maggior parte degli analizzatori impiegano 2 o piu` metodi per contare e differenziare gli stessi elementi cellulari

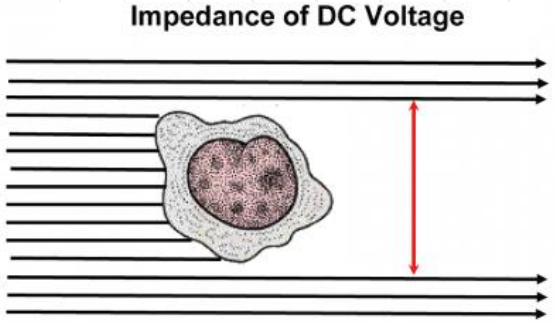


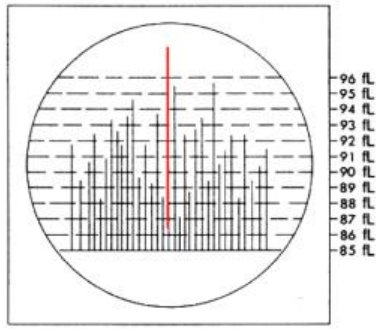
Referto strumentale ematologico: conteggi, curve di distribuzione, citogrammi

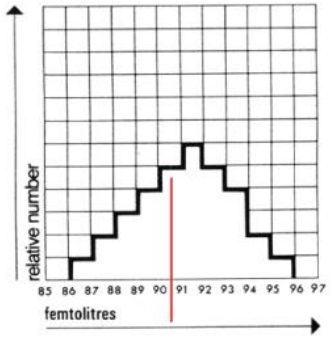
- Il metodo Coulter (Wallace Coulter, 1948), è in uso in molte piattaforme analitiche



- il metodo esegue il conteggio ed il dimensionamento delle cellule misurando le variazioni di resistenza elettrica quando una particella, posta in un diluente, passa attraverso aperture di piccole dimensioni
- La cellula funge da isolante e crea resistenza al percorso elettrico tra gli elettrodi posti ai lati dell'apertura stessa. Questa alterazione è rilevata in forma di impulso
- Il numero degli impulsi indica il conteggio delle particelle, la dimensione degli stessi è proporzionale al volume

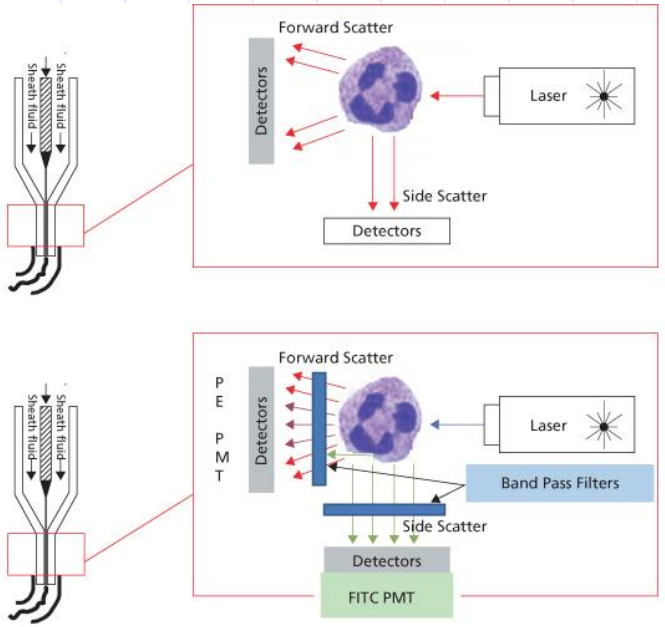


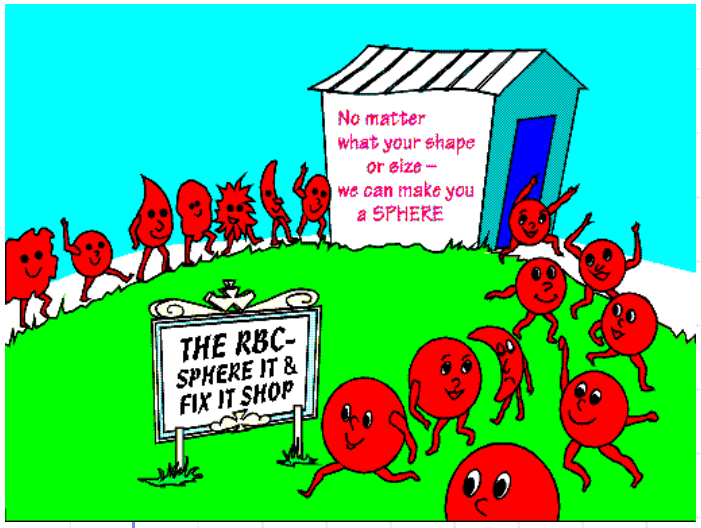


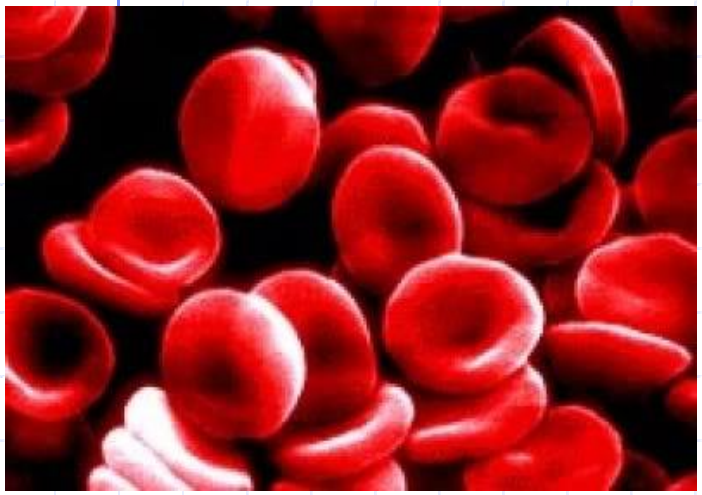


Misurazioni ottiche della luce

- misura dell' assorbimento della luce da parte delle cellule - in citochimica automatizzata mediante reazione citochimica specifica per l'enzima mieloperossidasi all'interno dei leucociti
- perdita di luce assiale (ALL) a 0°, misura inserita nelle analisi di VCSn
- scatter della luce, generalmente utilizzate le misure di scatter ad angolo ridotto rispetto al raggio laser (forward scatter – dimensioni cellulari) e in direzione ortogonale (side scatter – strutture interna alla cellula e soprattutto forma del nucleo e caratteriatiche dei granuli citoplasmatici)
- misure di fluorescenza, evidenziazione di RNA/DNA mediante coloranti fluorescenti, oppure anticorpi monoclonali specifici per determinati target (ad esempio, CD61 per la rilevazione PLT Abbott)

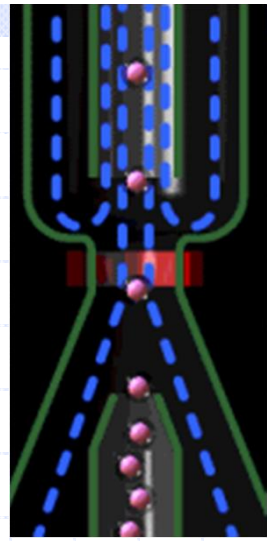


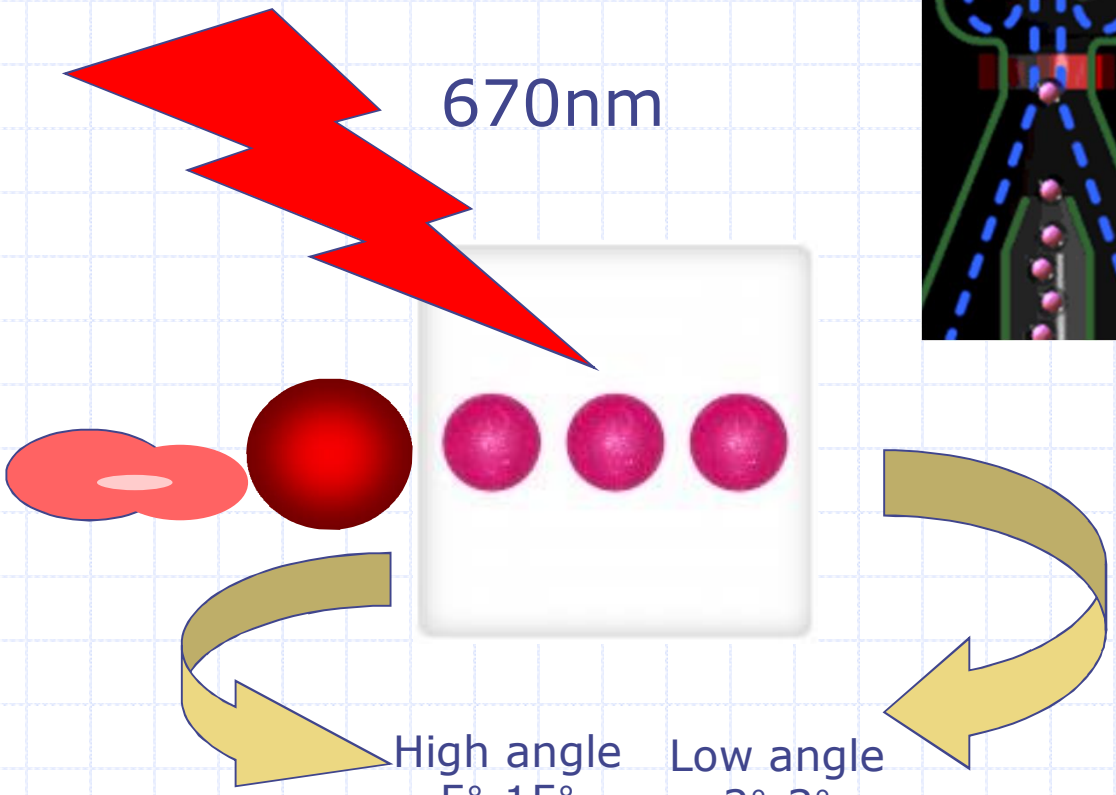




RBC

Analisi RBC





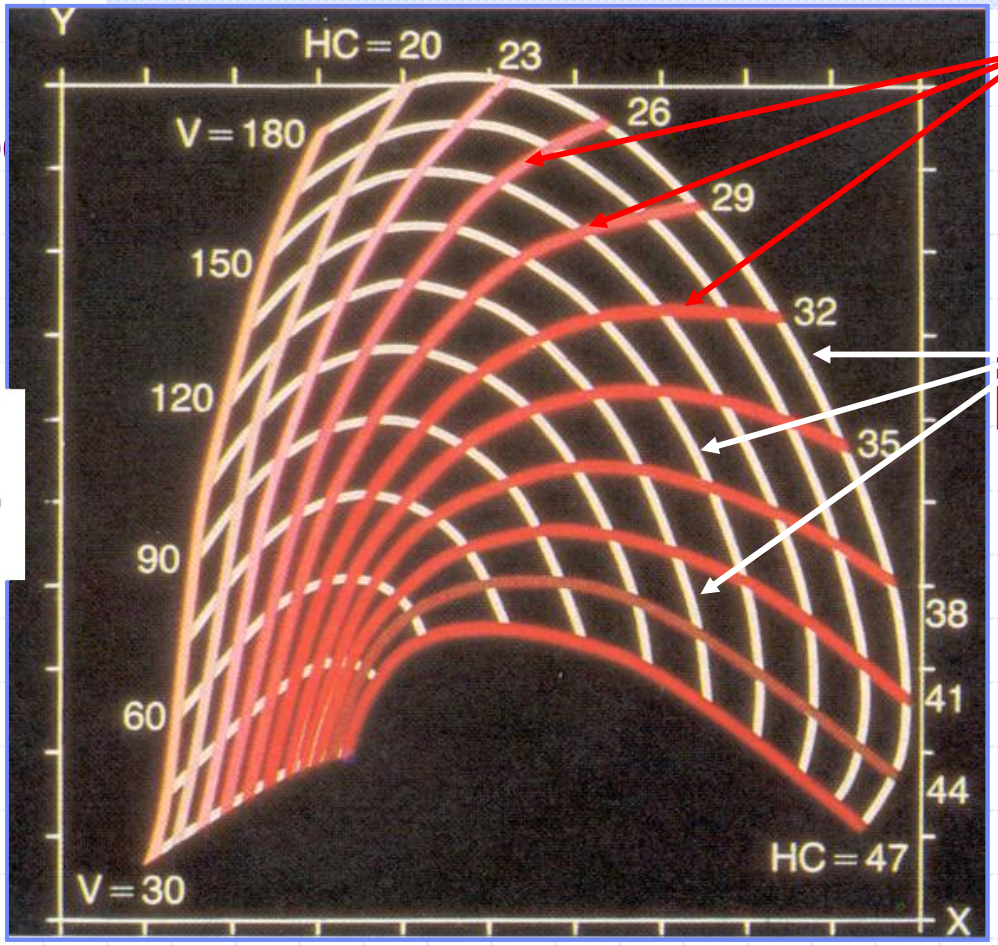
LE MAPPE ERITRO

blood 1986 68: 506-513

Accurate and independent measurement of volume and hemoglobin concentration of individual red cells by laser light scattering

N Mohandas, YR Kim, DH Tycko, J Orlik, J Wyatt and W Groner

Mappa MIE



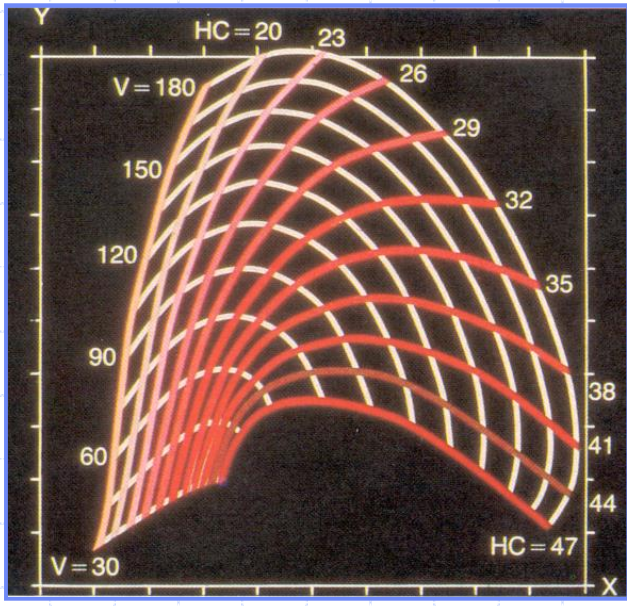
- La mappa RBC mostra graficamente la relazione, cellula per cellula, tra le misurazioni della dispersione della luce e le caratteristiche di volume (y) e di concentrazione di emoglobina (x)
- La griglia della mappa include volumi eritrocitari compresi tra 30 e 180 fL e concentrazioni emoglobiniche tra 19 e 49 g/dL ed è costituita da linee a medesimo volume ed HC
- In un campione normale la popolazione RBC è riportata al centro della mappa.

LE MAPPE ERITROCITARIE

MAPPE DI MIE

La mappa di Mie non è lineare e non consente una agevole valutazione visiva della distribuzione eritrocitaria.

Il sistema ne elabora una rappresentazione lineare, denominata *ñitogramma V/HCò* caratterizzata da nove aree distinte di morfologia eritrocitaria



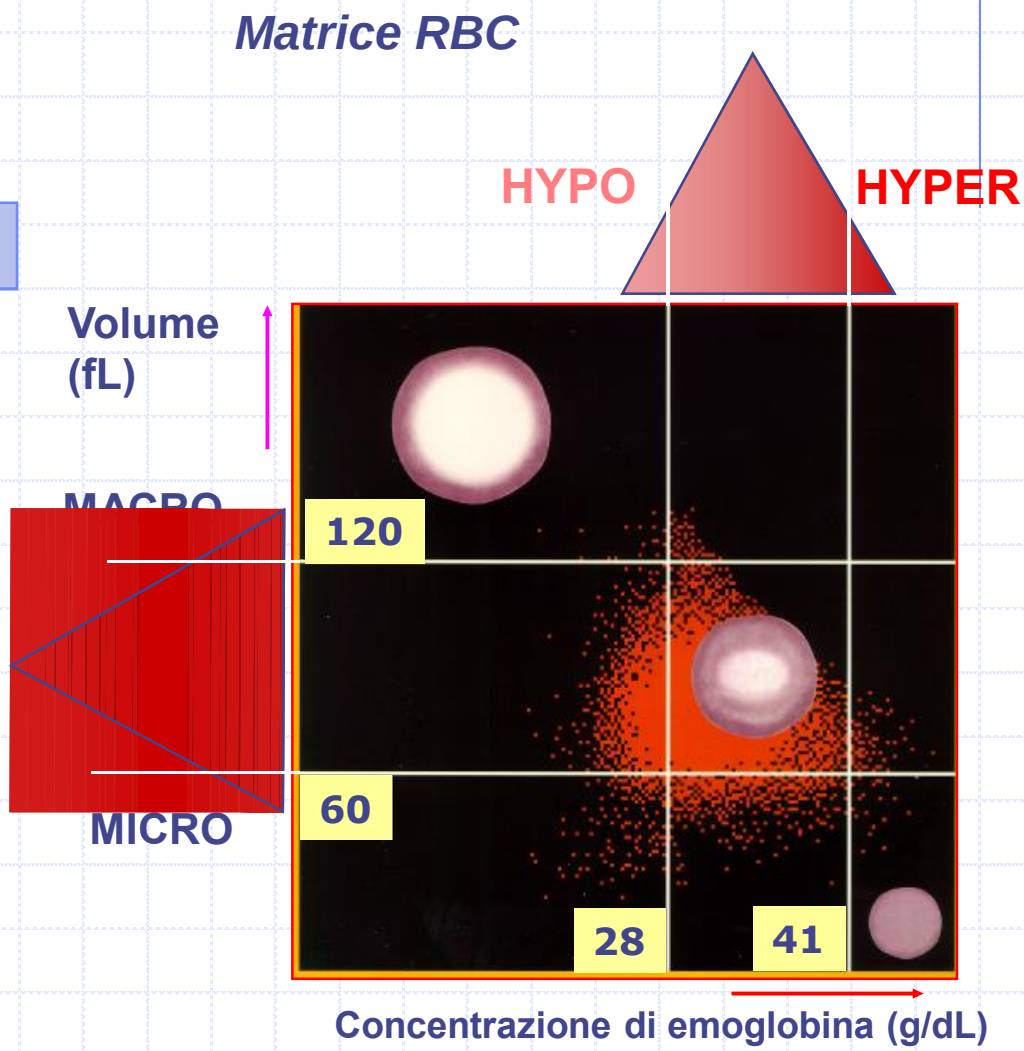
Analisi degli eritrociti

Parametri eritrocitari derivati

% hypo: carenza ferro, terapia EPO

% micro / % hypo: differenziazione di talassemia e deficit di ferro (>0,9 in tal)

%hyper: indicativo di sferocitosi



LE MAPPE ERITROCITARIE

PARAMETRI ERITROCITARI DERIVATI

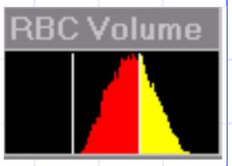
ISTOGRAMMA VOLUME RBC: rappresenta la distribuzione dei globuli rossi per volume della cellula.

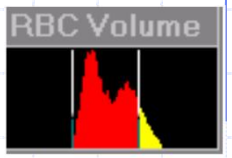
- intervallo tra 0 fL e 200 fL, discriminanti a 60 e 120 fL.

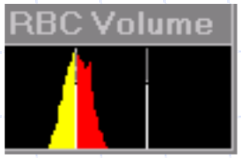
Vengono derivati dall'istogramma:

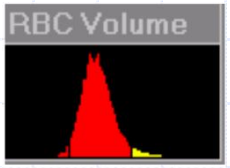
- MCV media della popolazione RBC;
- RDW coefficiente di variazione dell'istogramma. ("width of volume distribution")

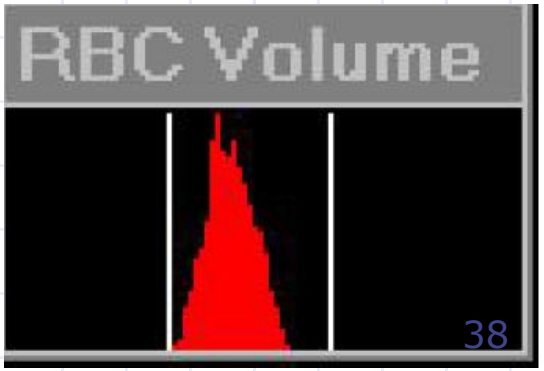
Flag morfologici RBC (alterazione della distribuzione volumetrica): anisocitosi (RDW), microcitosi (%MICRO), macrocitosi (%MACRO)





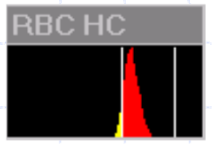


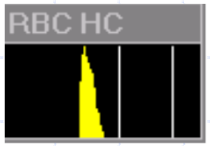


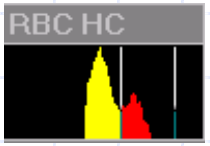


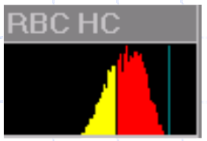
LE MAPPE ERITROCITARIE

PARAMETRI ERITROCITARI DERIVATI









ISTOGRAMMA CONCENTRAZIONE EMOGLOBINA RBC:

rappresenta la distribuzione dei globuli rossi per concentrazione cellulare di emoglobina.

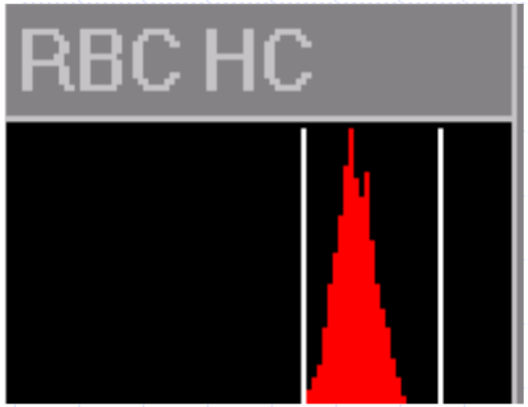
- Intervallo tra 0 g/dL e 50 g/dL, discriminanti a 28 e 41 g/dL.

Vengono derivati dall'istogramma:

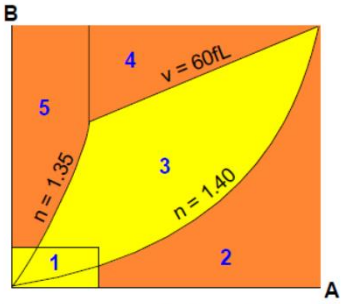
- CHCM mediana dell'istogramma HC RBC;
- HDW deviazione standard dell'istogramma.

("hemoglobin concentration distribution")

Flag morfologici RBC (alterazione della concentrazione di Hb): anisocromia (HDV >3.4 g/dL), ipocromia (%Ipo), ipercromia (%Iper)



Analisi integrata delle piastrine



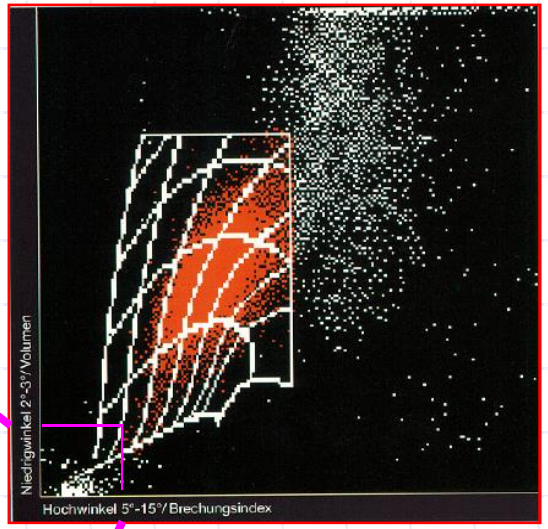
1 Area coperta dal citogramma disp. PLT
2 Frammenti eritrocitari
3 Grandi piastrine
4 Globuli rossi
5 Ombre eritrocitarie

asse x (**A**) high-angle (da 5° a 15°) dispersione della luce

asse y (**B**) low-angle (da 2° a 3°) dispersione della luce

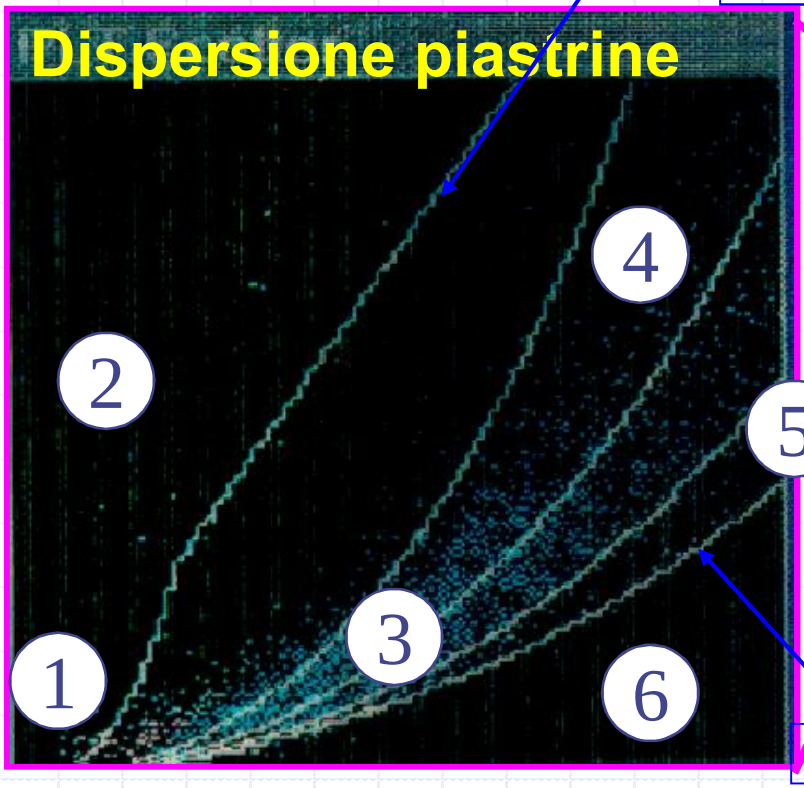
n = indice di rifrazione

v = volume cellulare



1,3500

2 - 3°

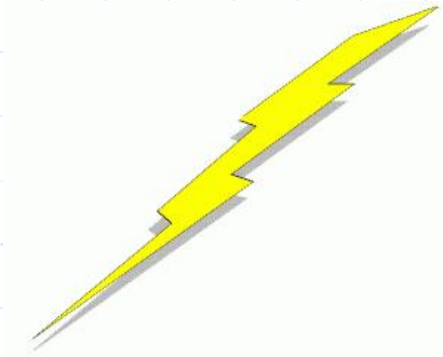


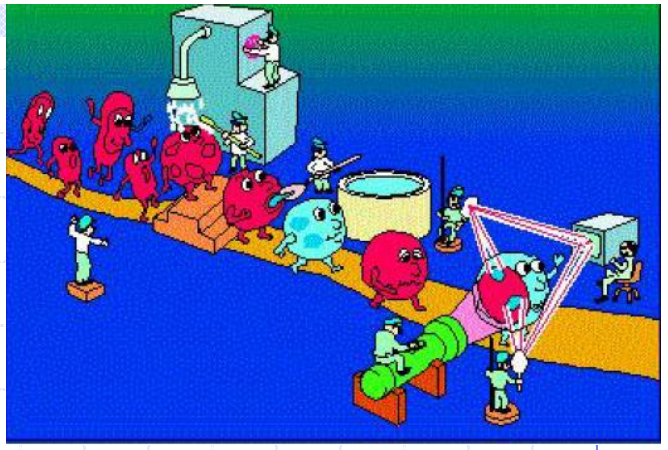
1,4000

5 - 15 °, indice di rifrazione (IR)

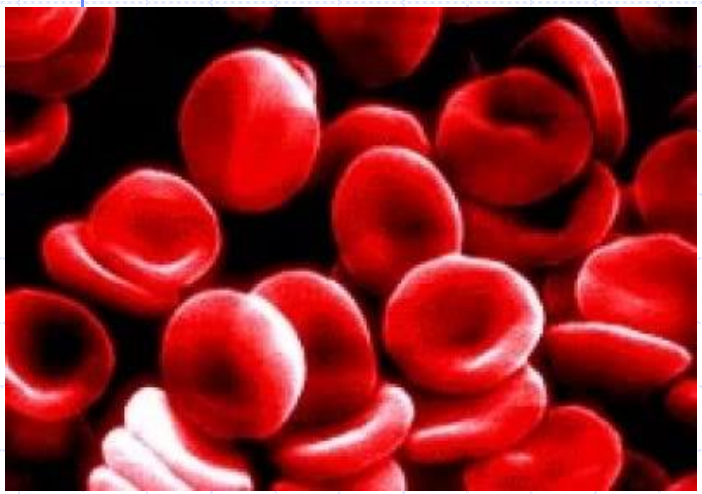
① **Detriti**
② **Ombre eritrocitarie**
③ **Piastrine fino a 20 fl**
④ **Piastrine grandi > 20 fl**
⑤ **Microciti**
⑥ **Frammenti Eritrocitari (>100000/μL)**

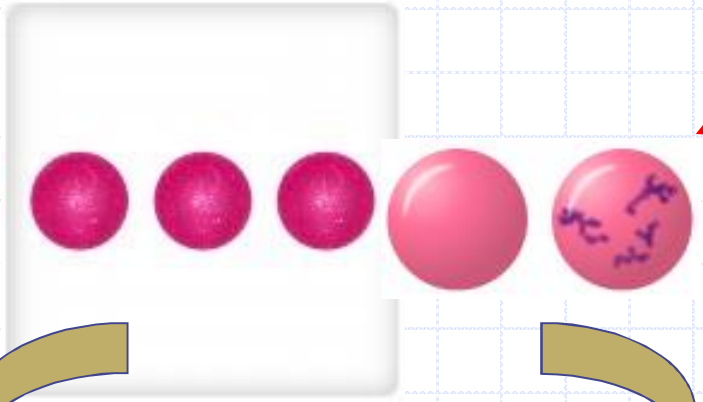
Analisi RETIC





Oxazina 750





High angle
5°-15°
CHCM

Low angle
2°-3°
MCV

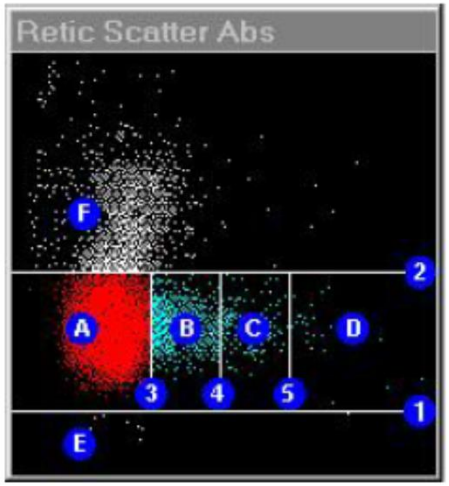
RBC DIL

Assorbanza (RNA)

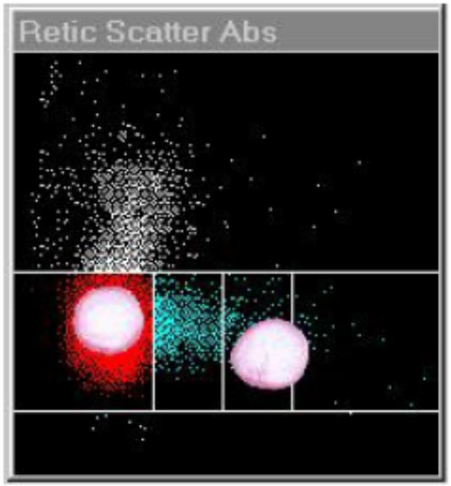
Il citogramma Scatter/Abs RETIC

Il citogramma Assorbimento scatter RETIC è la rappresentazione grafica delle misurazioni di assorbimento e light scatter: l'assorbimento ad alto guadagno (maturazione della cellula) viene tracciato lungo l'asse delle x e l'high-angle light scatter a basso guadagno (dimensioni della cellula) viene tracciato lungo l'asse delle y.

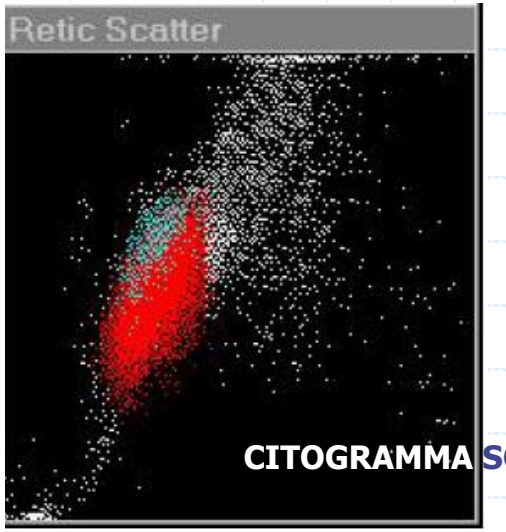
DENSITÀ



1 Soglia piastrine RTC
2 Soglia coincidenza RTC
3 Soglia RTC
4 Soglia RTC bassa/media
5 Soglia RTC media/alta
A RBC maturi
B Reticolociti a basso assorbimento
C Reticolociti ad assorbimento medio
D Reticolociti ad alto assorbimento
E Piastrine
F Eventi di coincidenza



ASSORBANZA

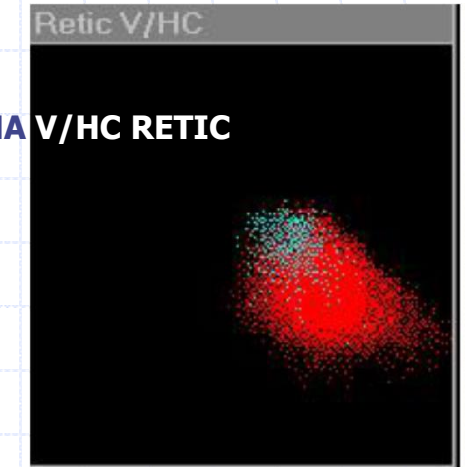


CITOGRAMMA SCATTER RETIC

Y volume
X HC

Mie scattering theory

CITOGRAMMA V/HC RETIC



X HC
Y volume

42

Analisi RETIC

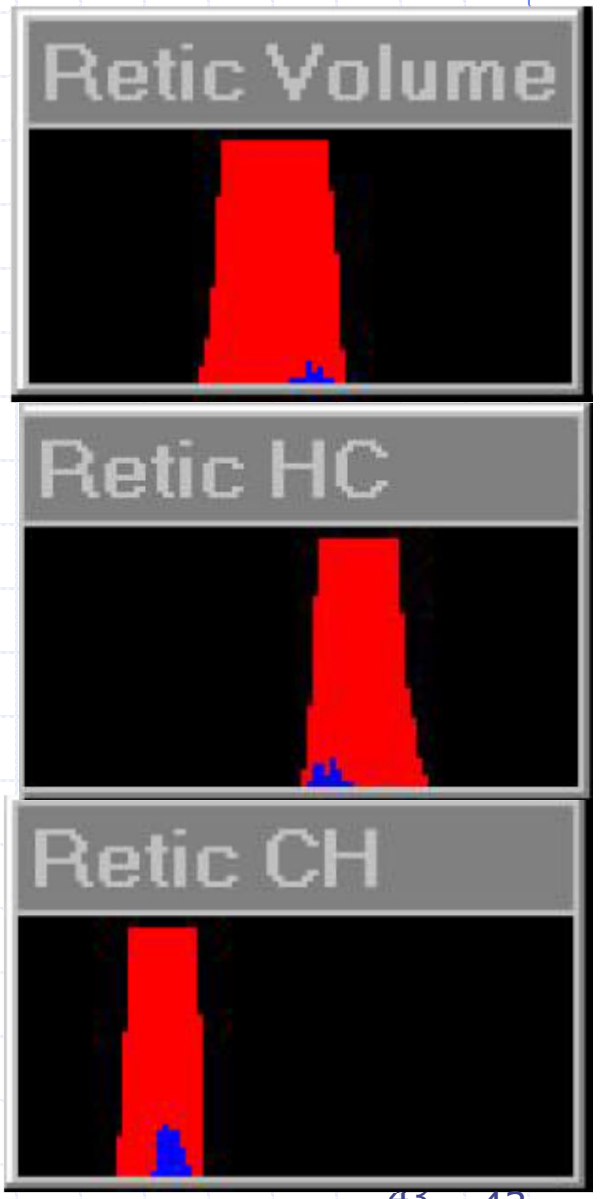
MCVr: media dell'istogramma Volume RETIC per la popolazione di reticolocit. MCV/MCVr, 24,3% in media

RDWr: coefficiente di variazione (CV) dell'istogramma Volume RETIC (canali da 15 a 99) per la popolazione di reticolociti

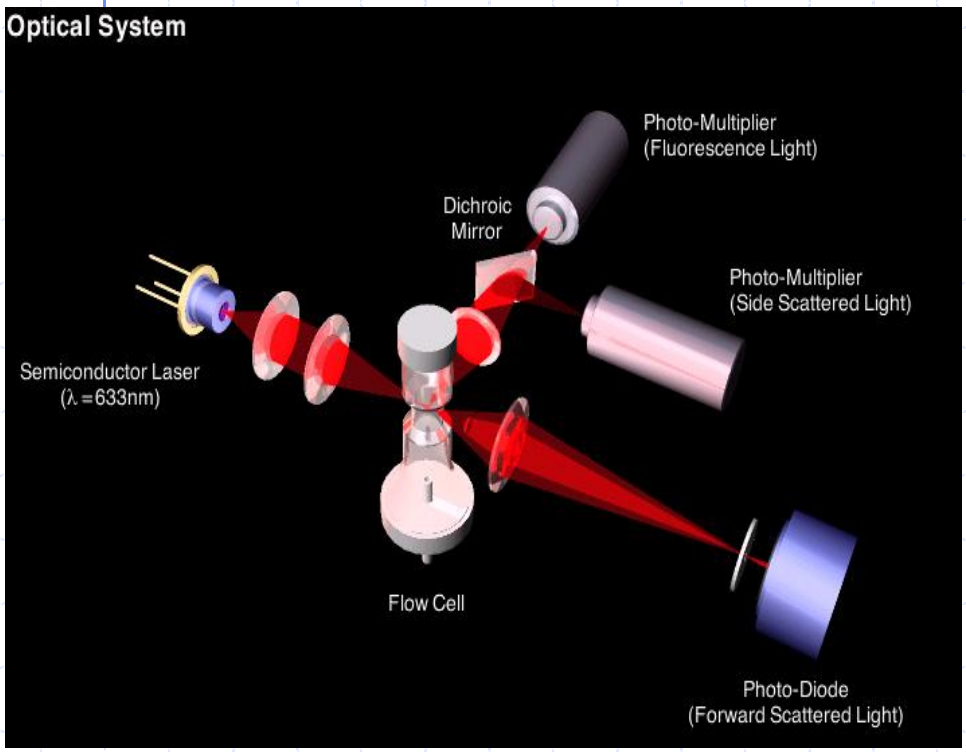
CHCMr: la media dell'istogramma HC RETIC per la popolazione di reticolociti: CHCM/CHCMr, -16,7% in media

HDWr: la deviazione standard (DS) dell'istogramma HC RETIC per la popolazione di reticolociti

CHr: media dell'istogramma CH RETIC per la popolazione di reticolociti; nei soggetti sani CHr = 28,6pg (DS 2,46)



Fluorescenza in Citometria a Flusso

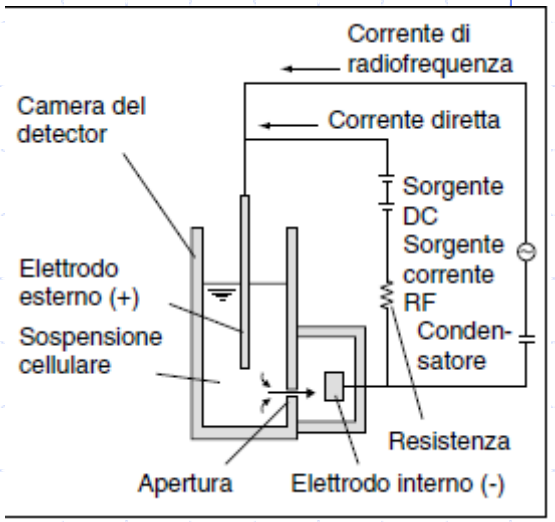


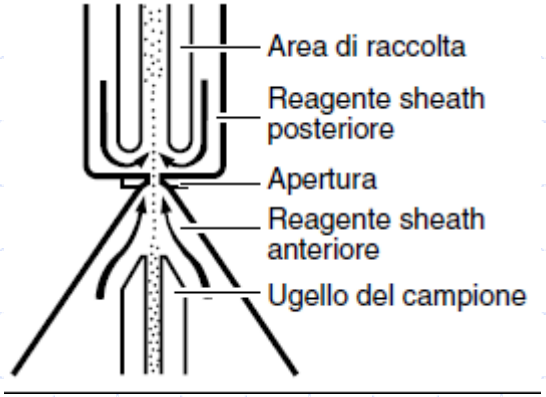
XE5000 illumina il campione con un Laser a semiconduttori (633 nm) e separa le cellule utilizzando i seguenti segnali:

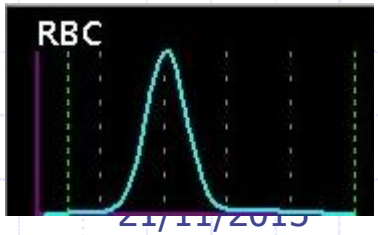
- ✓ Forward Scatter- Volume cellulare
- ✓ Side Scatter- Struttura interna
- ✓ Side Fluorescence - Quantitativo DNA/RNA

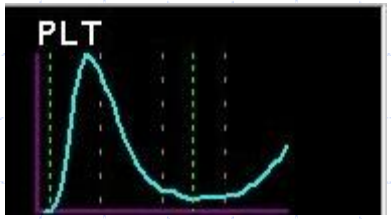
Conteggio RBC/PLT

- ✓ Canale specifico RBC/PLT, rilevazione RF/DC (Resistivo con Focalizzazione Idrodinamica)
- ✓ Il metodo RF/DC rileva le dimensioni cellulari dalle variazioni della resistenza alla corrente diretta e descrive la densità della struttura interna dalle variazioni della resistenza alla radiofrequenza.
- ✓ Le dimensioni degli impulsi permettono di costruire scattergram e distribuzioni dimensionali
- ✓ Secondo conteggio su canale RET, mediante lettura ottica con laser

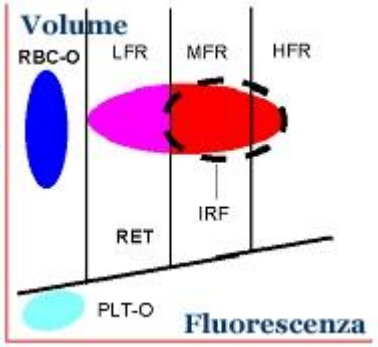




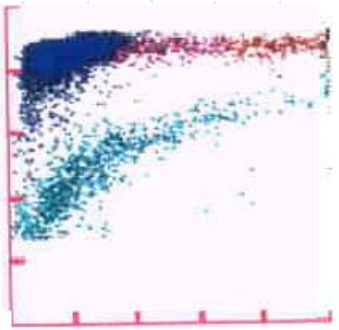




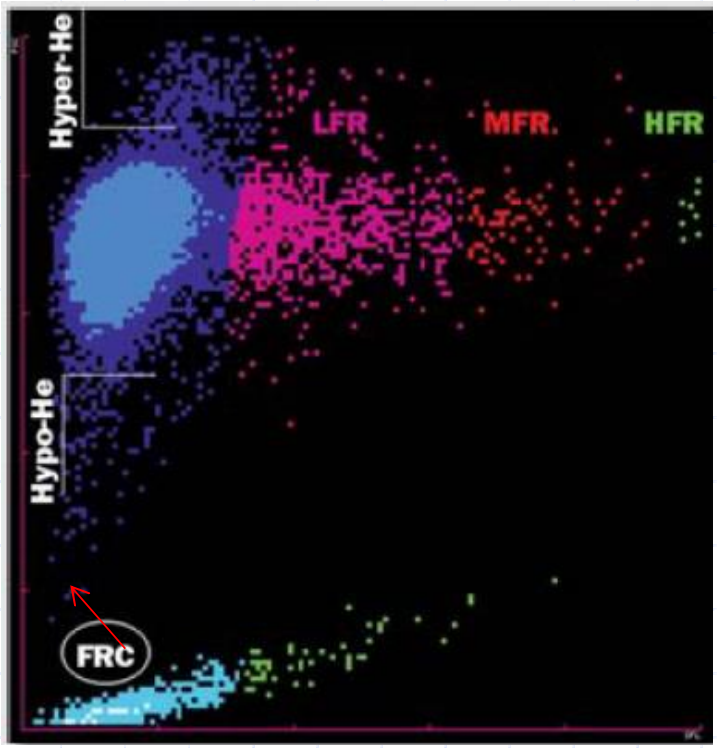
RET

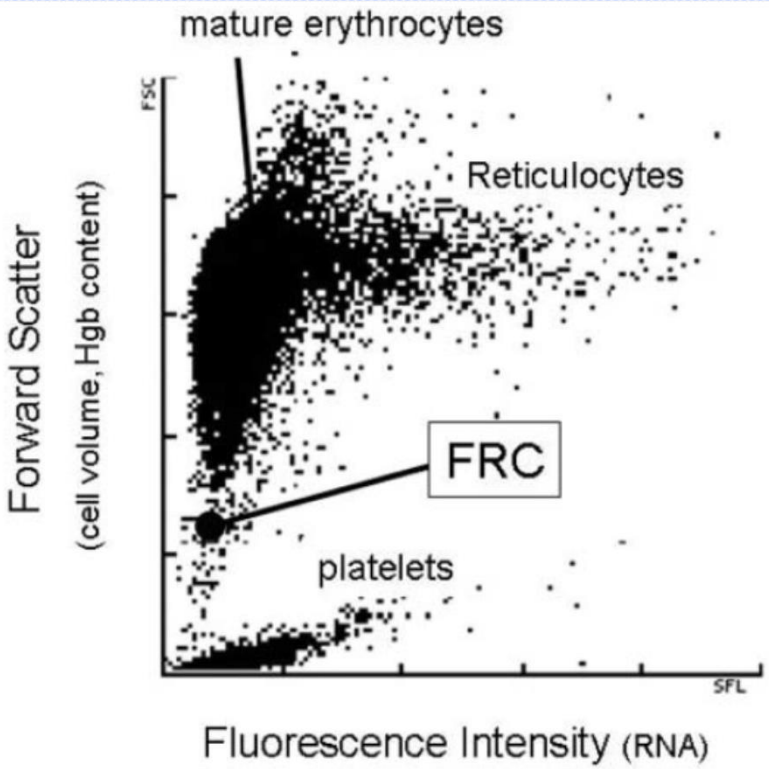


PLT-O



Analisi Reticolociti

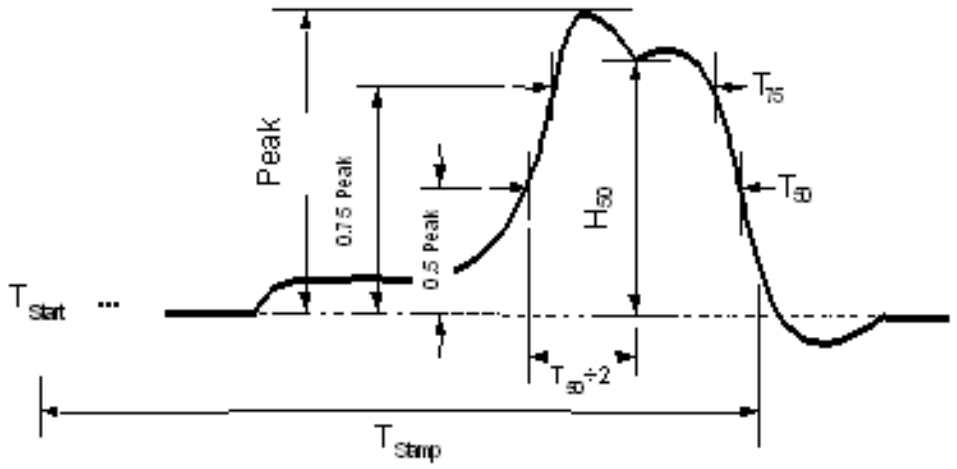




- L'analisi RET (citometria in fluorescenza) classifica e analizza eritrociti, piastrine, reticolociti e sottogruppi RET
- Colorazione con un reagente fluorescente (polymethine) e scattergram con forward scatter in asse Y (volume) ed intensità di fluorescenza laterale in asse X

- Studio della popolazione RBC e specifiche popolazioni - %IPO (Hypo-He), %IPER (Hyper-He), % microciti e macrociti, contenuto in Hb (RBC-He), frammenti eritrocitari (FRC) in % e #
- Per RET si hanno 3 frazioni maturative a differente intensità di fluorescenza e calcolati parametri relativi al contenuto RET di Hb (RET-He, formalmente RET-Y)

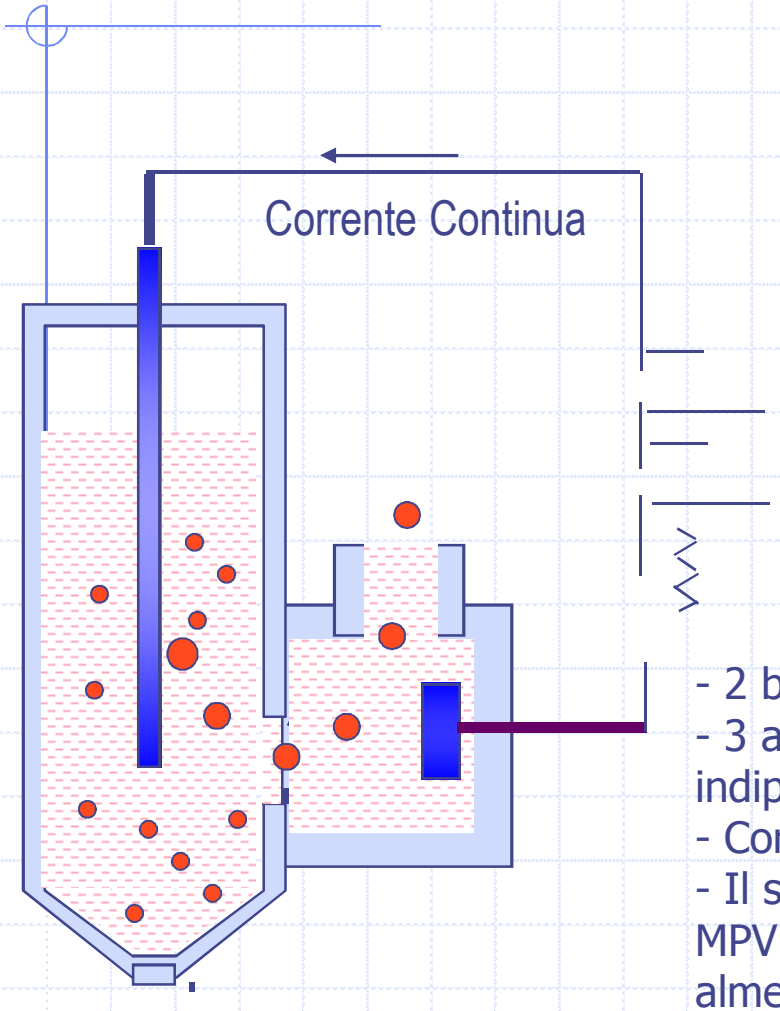
- Conteggio di WBC, RBC, PLT e parametri derivati
- Basato sul principio Impedenziometrico Coulter, con algoritmi di analisi per una migliore classificazione volumetrica delle cellule
- Tecnologia "Digital Pulse Processor" che fornisce misure di più parametri su ogni evento cellulare all'interno della cella

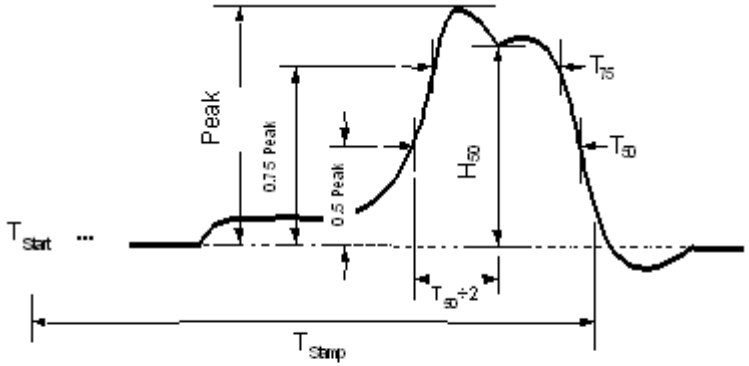


DxH Digital Pulse Characteristics

Conteggio e dimensionamento RBC, PLT, WBC

Impulso Elettrico



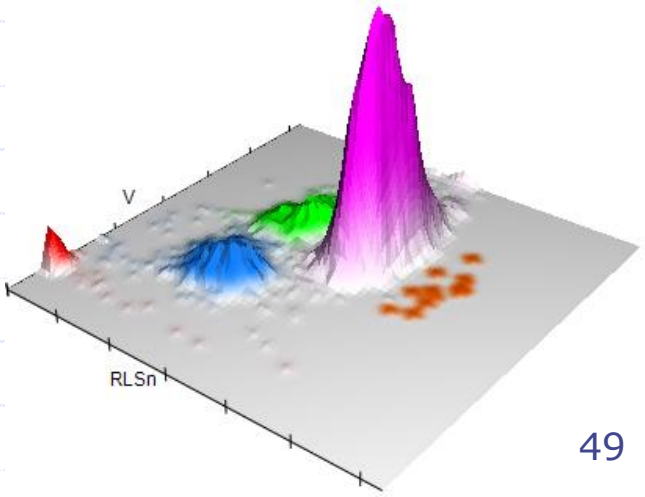


DxH Digital Pulse Characteristics

- 2 bagni separati per WBC e RBC/PLT
- 3 aperture distinte che funzionano come sistemi indipendenti e che utilizzano il metodo Coulter
- Correzione della coincidenza
- Il sistema analizza i dati per WBC, RBC, MCV, RDV e MPV confrontando i dati delle 3 aperture e verifica che almeno 2 aperture abbiano fornito dati simili

Flow Cytometric Digital Morphology

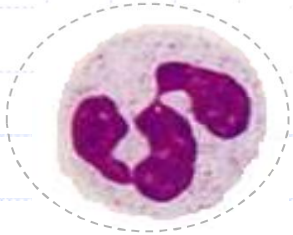
- Determinazione diretta delle 5 sottopopolazioni
- Analisi delle cellule allo stato nativo attraverso un unico canale di rilevazione.
- Tecnologia FCDM applicata per l'analisi di **formula** leucocitaria, **reticolociti**, **NRBC e PLT**



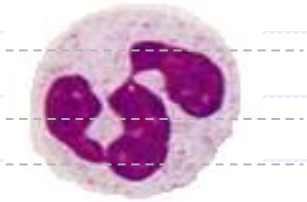
Flow Cytometric Digital Morphology

Misurazione diretta di 7 parametri su ciascuna cellula

Dimensione Cellulare (Principio Coulter)



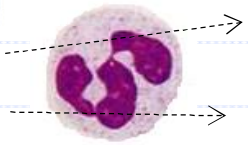
Caratteristiche cellulari interne, rapporto nucleo/citoplasma, densità nucleare e composizione chimica (Radiofrequenza→ conduttività)



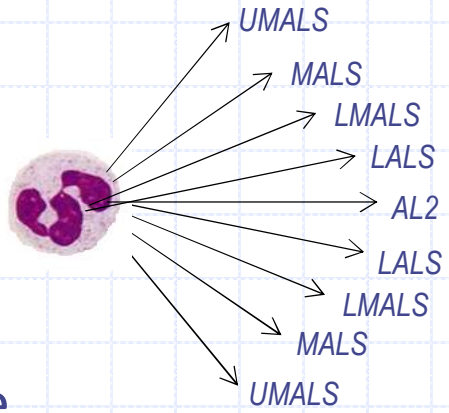
Flow Cytometric Digital Morphology

Misurazione diretta di 7 parametri su ciascuna cellula

Le caratteristiche cellulari sono studiate da **5 misure di scatter di luce laser**



- UMALS (Scatter ad angolo medio-alto)
- MALS (Scatter ad angolo medio)
- LMALS (Scatter ad angolo medio-basso)
- LALS (Scatter ad angolo basso)
- AL2 (perdita di luce laser)



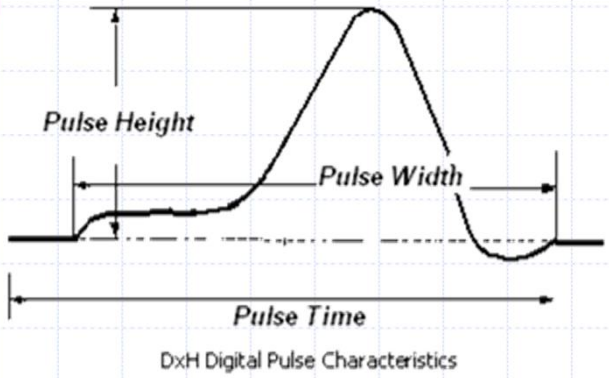
Ciascuno scatter è in grado di vedere ed evidenziare caratteristiche diverse della morfologia cellulare.

Flow Cytometric Digital Morphology

Misurazione diretta di 7 parametri su ciascuna cellula
(WBC, RET, NRBC, PLT)

Misurazione diretta
di 7 parametri
su ciascuna cellula

analisi digitale del segnale di ciascuna misurazione: ulteriori 4 determinazioni per evento

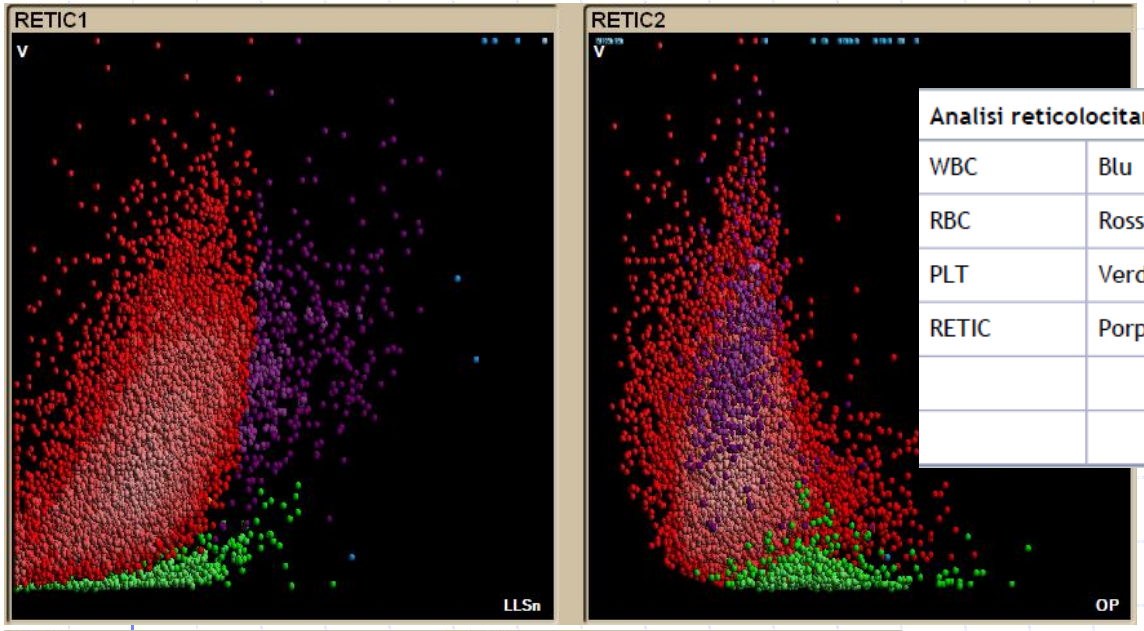


DxH Digital Pulse Characteristics

Integrazione ed analisi del fattore tempo di ciascuna determinazione

Per un totale di 29 misurazioni
per singolo evento (cellula)

Reticolociti



Reagenti specifici Determinazione DIRETTA con CANALE DEDICATO

- Methylene-blue staining (RNA), fissaggio in forma sferica
- 28 parametri cellulari nel modulo RETIC: impedenza corrente diretta (volume-fenomeno Coulter), opacità ad RF (conduttività) e VCSn
- Differenti paramentri cellulari: HLR, IRF, MRV

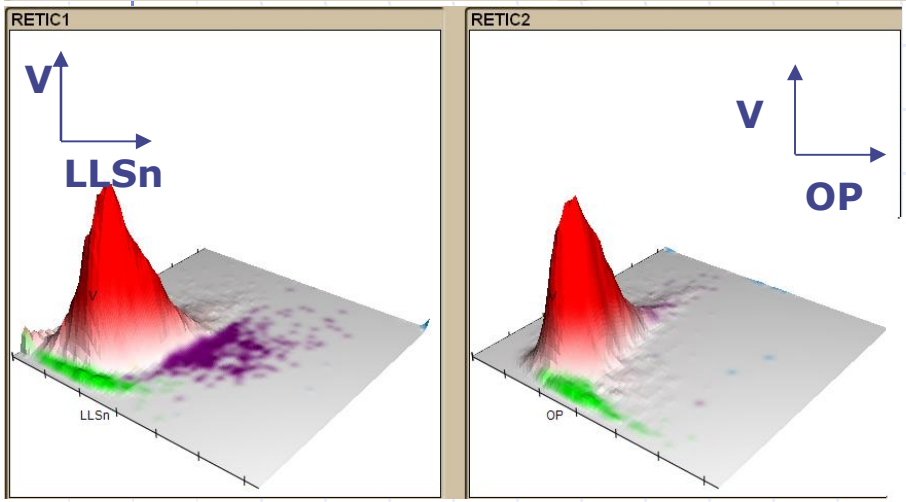
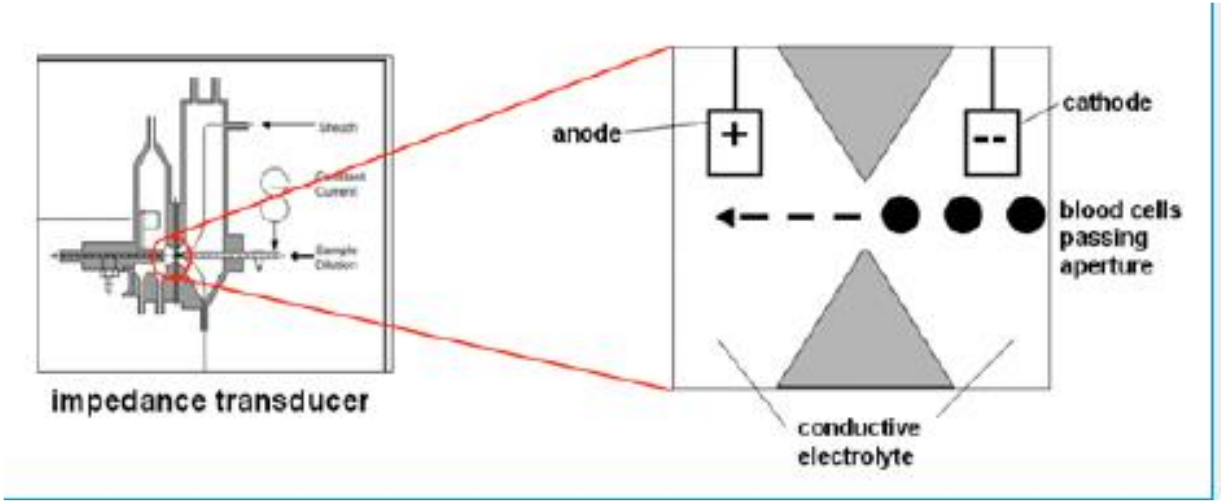


Table 4 Blood count parameters (mean value and SD) in the different groups of patients

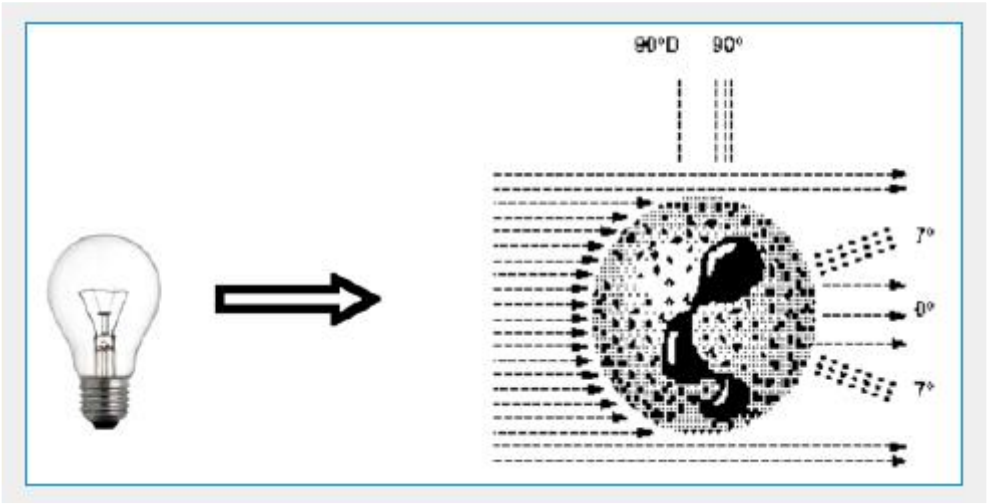
	IDA	IDA treat	CKD
RBC 10^{12}/l	4.10 (0.39)	4.27 (0.41)	3.61 (0.56)
HGB g/l	99 (9)	109 (11)	108 (12)
MCV fl	73.9 (7.4)	78.6 (7.7)	91.2 (7.6)
RDW %	17.2 (2.5)	18 (3.9)	16.4 (2.1)
RET %	1.41 (0.45)	2.8 (0.93)	1.9 (0.83)
IRF unit fraction	0.30 (0.07)	0.38 (0.07)	0.40 (0.08)
MRV fl	106.9 (11.6)	111.3 (18.4)	128.9 (11.1)

RBC, red blood cells; HGB, haemoglobin concentration; MCV, mean corpuscular volume; RET, reticulocyte percentage; IRF, immature reticulocyte fraction (expressed in unit fraction); MRV, men reticulocyte volume.

J Clin Pathol 2011;**64**:159–163. doi:10.1136/jcp.2010.082669



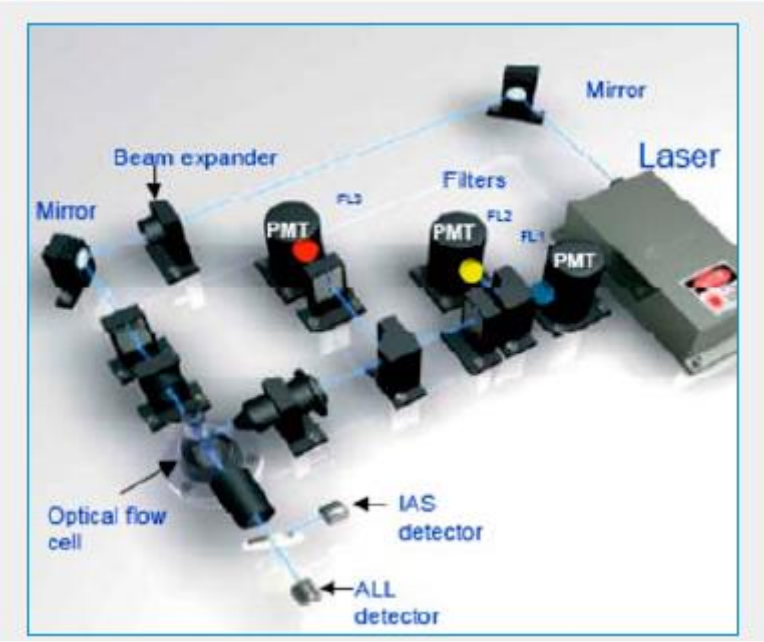
- CELL-DYN Sapphire utilizza il metodo per impedenza per contare RBC e, come metodo secondario, le PLT
- Il metodo viene utilizzato anche per il dimensionamento di RBC e PLT (volume cellulare)
- Metodo per impedenza secondo principio di Coulter
- Impiego della tecnologia di focusing idrodinamico (un cilindro liquido circonda il campione diluito e si sposta con esso in forma di flusso laminare)



- metodo di analisi ottico per contare e differenziare WBC, metodo primario per PLT; metodo secondario per RBC e per ulteriori parametri RBC. Impiego della tecnologia di focusing idrodinamico
- Il metodo ottico utilizza una sorgente luminosa monocromatica a luce blu e lunghezza d'onda pari a 488 nm; la stessa sorgente luminosa è impiegata per le misurazioni in fluorescenza
- 4 rilevatori di scatter a differenti angoli rispetto al raggio di luce con informazioni relative a diversi aspetti cellulari; la misura della luce depolarizzata permette la separazione specifica degli eosinofili

Table 1.1 Four angles of light scatter detection in CELL-DYN Sapphire

Angle	Designation	Represents
0°	axial light loss (ALL)	cell size
7°	intermediate angle scatter (IAS)	cell complexity (internal structure)
90°	polarized side scatter (PSS)	nuclear segmentation (lobularity)
90°D	depolarized side scatter (DSS)	eosinophilic granules



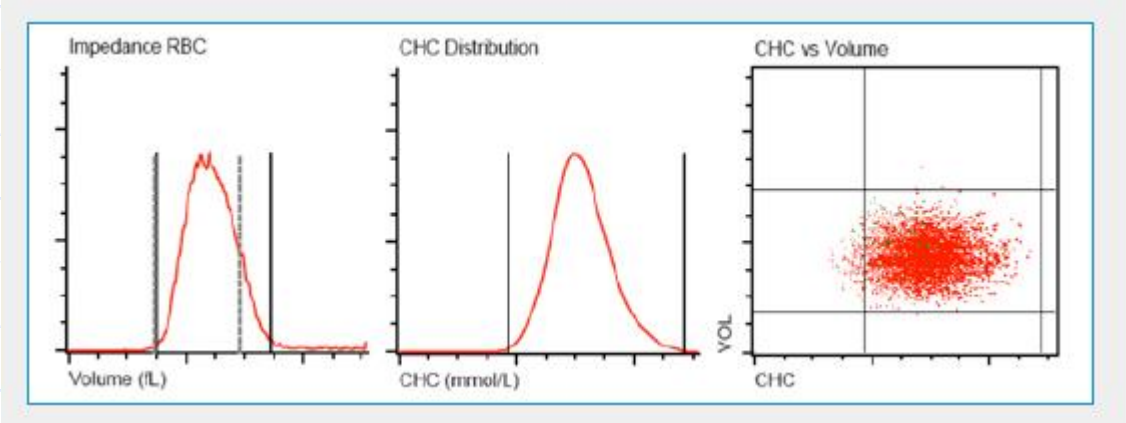
- Il metodo di analisi per fluorescenza viene impiegato per la rilevazione di strutture intracellulari (RNA e DNA) e per l'applicazione di metodi in immunofluorescenza, definibili dall'operatore
- Il metodo utilizza la medesima sorgente luminosa monocromatica già impiegata per il metodo ottico
- Vengono impiegati 3 rilevatori di fluorescenza (fotomoltiplicatori) a differenti lunghezze d'onda

Fluorescence detector	Wavelength	Application
FL1	530 nm (green)	reticulocytes FITC-labeled antibodies
FL2	570 nm (orange)	PE-labeled antibodies
FL3	630 nm (red)	white blood cell viability, NRBC

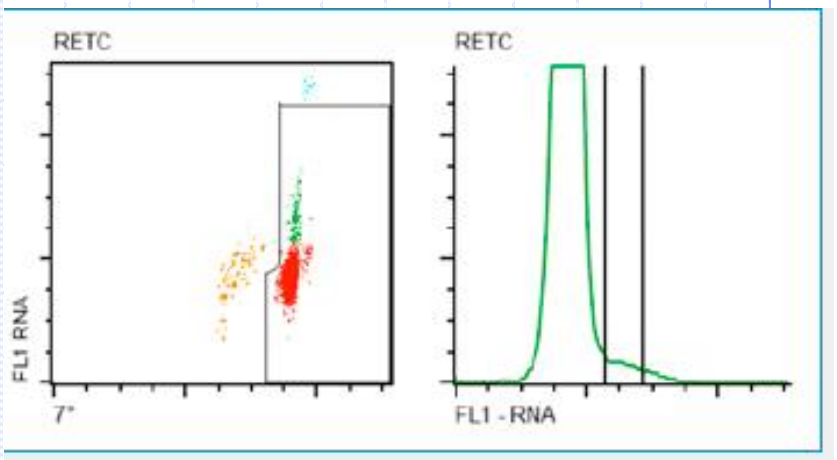
Conteggio RBC

- ✓ RBC sono sottoposti a sfericizzazione isovolumetrica
- ✓ Conteggio RBC mediante 2 metodi, in impedenza (metodo primario) e con metodo ottico

- ✓ La distribuzione volumetrica RBC (dati da impedenza) viene convertita in un istogramma, con 2 discriminanti dinamici, utilizzati per il calcolo di MCV, e 2 discriminanti fissi a 60 e 120 fL
- ✓ Il metodo ottico raccoglie informazioni da 3 angoli di scatter di luce, 0°, 7° e 90°
- ✓ I segnali di scatter permettono di calcolare, per ogni singola cellula ed applicando i principi della teoria di Mie, il volume e la concentrazione di emoglobina cellulare (CHC)
- ✓ Costruzione di un istogramma di distribuzione della concentrazione di Hb cellulare con 2 discriminanti fissi (CHC < 28 g/dL e >41 g/dL) e di un citogramma volume (Y) e CHC (X) con gli stessi discriminanti per V e CHC



Conteggio RET



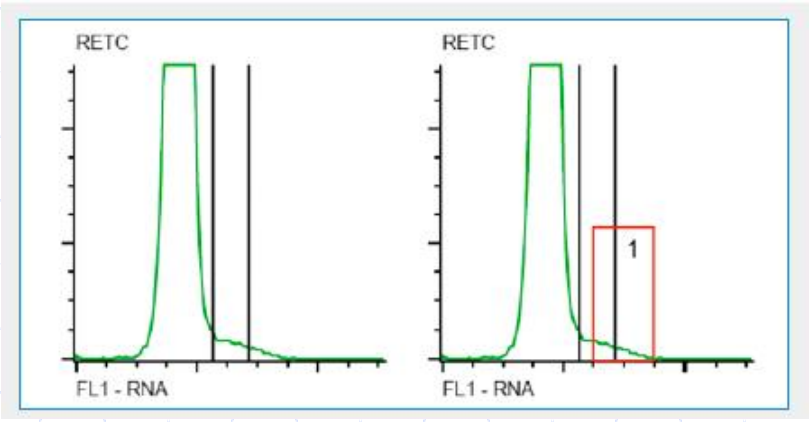
✓Utilizza una sub-diluizione del campione RBC e un reattivo fluorescente per RNA

✓ Conteggio ed analisi (FL1) dei dati di fluorescenza e di scatter della luce a 0°, 7° e 90°

✓Vengono costruiti 1 scatterplot e 1 istogramma; il primo (FL1 RNA/7°) separa RBC (rosso), PLT (arancio) e WBC (blu); il secondo separa RBC (bassa fluorescenza), RET e la frazione RET immatura (ad elevata fluorescenza – a destra rispetto al discriminante maggiore).

In entrambi i casi mediante discriminanti dinamici

✓Calcolo di IRF



✓La presenza di un picco in regione 1 suggerisce la presenza di infestazione da plasmodi malarici

Messaggi da portare a casa

- ✓ Che parametri guardare in un referto strumentale
  - ✓ i parametri numerici, MCV, RDW, reticolociti, flags
  - ✓ MCH e MCHC, come indicatori della qualità del campione analizzato (emolisi, agglutinazione RBC, torbidità del plasma) e del corretto funzionamento dell'analizzatore; MCHC nella HS
    - ✓ CHCM (misurazione diretta ottica) → non risente di opacità del plasma, di Hb libera, di agglutinati
  - ✓ gli istogrammi di distribuzione cellulari (%Micro, %Macro)
  - ✓ conoscere bene il vostro analizzatore e le caratteristiche dei citogrammi e scegliere quelli piu' informativi rispetto alla vostra popolazione di riferimento
  - ✓ tra i parametri "solo di laboratorio", utilizzare clinicamente (e refertare) solo quelli sostenuti dalla maggiore evidenza scientifica
    - ✓ CHr e %IPO, %IPER (RET-He, RBC-He)
    - ✓ %Micro/%Ipo ratio
    - ✓ frazione immatura dei RET (IRF)
    - ✓ IPF

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Table A. Summary of the strength of evidence addressing Key Questions

Key Question	Strength of Evidence	Summary, Comments, and Conclusions
Key Question 2. What is the diagnostic test accuracy of newer markers of iron status as a replacement for or an add-on to classical laboratory markers?	Low/ Insufficient (depending on the test comparisons, study populations, or test performance outcomes)	• Among adult HD CKD patients, there is a low level of evidence that: – CHr has similar or better overall test accuracy compared with TSAT or ferritin to predict a response to IV iron treatment. Data from two studies suggest that CHr (with cutoff values of <27 or <28 pg) has a better sensitivity and specificity in predicting iron deficiency than classical markers (TSAT <20 or ferritin <100 ng/mL). – %HYPO has similar or better overall test accuracy compared with TSAT, and better overall test accuracy compared with ferritin to predict a response to IV iron treatment. Data suggest that %HYPO (with cutoff values of >6% or >10%) has a better sensitivity and specificity to predict iron deficiency (as defined by a response to IV iron treatment) than classical markers (TSAT <20% or ferritin <100 ng/mL). – sTfR has a similar test performance compared with classical markers (TSAT or ferritin) to predict a response to IV iron treatment. • There is insufficient evidence regarding: – Test performance of newer markers of iron status as an add-on to older markers. – Test performance comparing ZPPand hepcidin to predict a response to IV iron treatment in adult HD CKD patients. – Test performance comparing newer with classical laboratory markers to predict a response to IV iron treatment, in adult PD CKD and ND CKD patients, and in pediatric CKD patients.

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Table B. Research gaps and suggestions for future research

Key Question	Research Gaps	Suggestions for Future Research
Key Question 2. What is the diagnostic test accuracy of newer markers of iron status as a replacement for or an add-on to classical laboratory markers?	Insufficient evidence for the test performance of newer markers of iron status as an add-on to older markers	• It is important to use an independent reference standard when assessing the test performance. See "Cross-cutting issues" for the research gaps for establishing a reference standard for iron deficiency.
	Many existing studies are at a high risk of bias, limiting their utility in informing clinical practice	• General principles for the design of studies of diagnostic tests include the use of an appropriate reference standard, adequate description of the index and reference tests, blinded interpretation of test results, and independence of the index and reference standard tests.[48] • Studies assessing diagnostic accuracy should instead aim to enroll patients representative of the spectrum of disease typically seen in clinical practice. • Future studies should provide details about the study base and sampling methods.

GRAZIE PER LA VOSTRA PAZIENZA