Apport diagnostique des nouveaux indices érythrocytaires dans l'anémie

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Modern multichannel instrument technology

	Electrical impedance	Radiofrequency conductivity	Light scatter	Reticulocyte counting
Abbott (CELL-DYN Sapphire)	x	x	x	Fluorescent colorant Cyanine dye
ABX (Pentra-120)	x		x	Fluorescent colorant Thiazole orange
Siemens (ADVIA)			x	Oxazine 750 (non fluorescent)
Beckman coulter (LH series, DxH 800)	x	x	x	New methylene blue (non fluorescent)
Sysmex (XE-2100)	x	x	x	Fluorescent colorant Polymethine

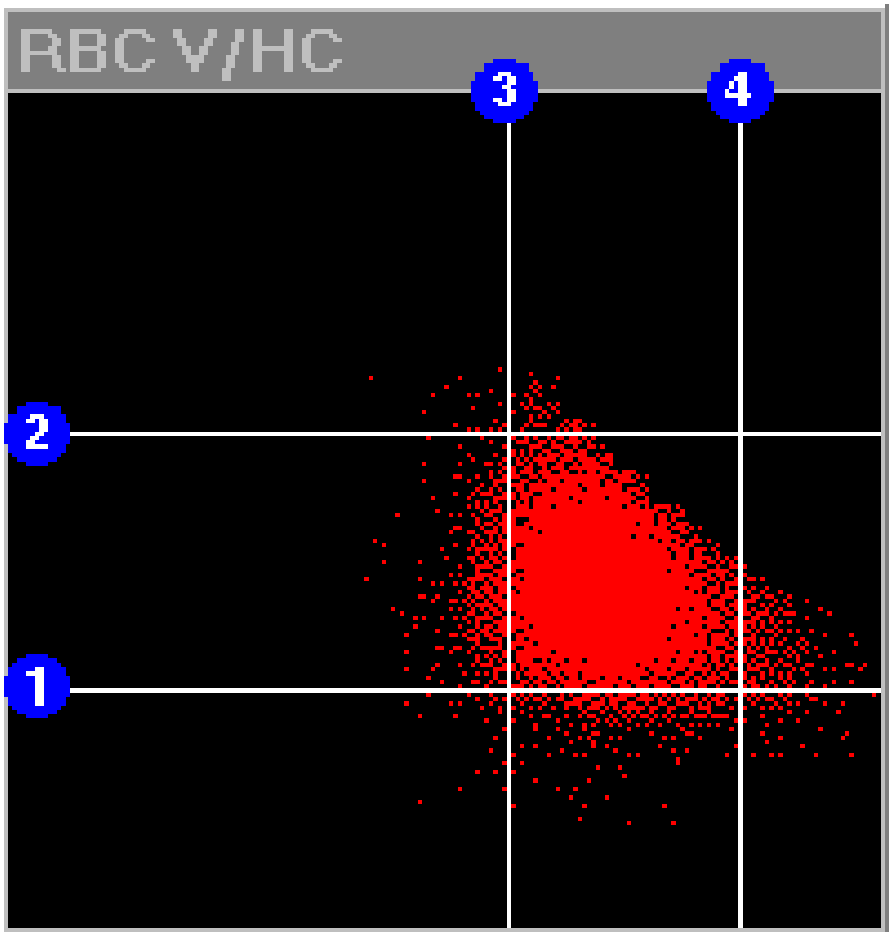
- New RBC and reticulocyte parameters

Parameter	Instrument	Clinical use	Ref
RBC extended parameters **%Hypo, %Hyper** **%Micro, %Macro; MicroR, MacroR** **LHD%, MAF**	ADVIA 2120 XE 2100 Cell-Dyn Sapphire LH 750	Å Restricted erythropoiesis (iron deficiency, beta thalassemia, ACD) Å Latent iron deficiency Å Hereditary spherocytosis	Bovy 2005 Urrechaga 2009, 2011 Maconi 2009 Piva 2010 Ermens 2012, Osta 2012 Rooney 2014, Ng 2014
Immature reticulocyte fraction **IRF**	ADVIA 2120 XE 2100 Cell-Dyn Sapphire Pentra 120 DX LH 750	Å Classification of anemias Å Early identification of BM regeneration/engraftment Å Early monitoring of response to treatment in anemia	Buttarello 2002 Torres Gomez 2001, 2003 Noronha 2003
Mean reticulocyte volume **MRV, MCVr**	ADVIA 2120 Pentra 120 DX LH 750	Å Diagnosis of iron-deficient erythropoiesis Å Early monitoring of response to treatment in anemia Å Early signs of erythropoietic recovery Å Epo abuse in sports Å Hereditary spherocytosis	D'Onorfio 1995 Brugnara 1998 Cappelletti 2006 Mullier 2011 Morkis 2014 Lazarova 2014
Reticulocyte Hb content (equivalent) **CHr, Ret He**	ADVIA 2120 XE 2100	Å Restricted erythropoiesis (iron deficiency, chronic inflammation) Å Latent iron deficiency Å Monitoring response to Fe or Epo treatment in CKD	Thomas 2002, 2005 Buttarello 2004 Fishbane 2001 Ullrich 2005 Brugnara 2006 van Santen 2011 Joosten 2013
Reticulocyte distribution width **RDWR-CV (SD), RDWr**	ADVIA 2120 LH 750	Å Restricted erythropoiesis (iron deficiency, beta thalassemia, ACD) Å Hereditary spherocytosis	Oustamanolakis 2011 Lazarova 2014
Other **RSf (red cell size factor)** **MSCV (mean sphered red cell volume)**	LH 750	Å Iron deficiency with or without chronic inflammation Å Hereditary spherocytosis	Urrechaga 2010, Ng 2014 Broseus 2010 Lazarova 2014 Liao 2014

Not standardized; reference intervals: method-dependent

The RBC Method ADVIA

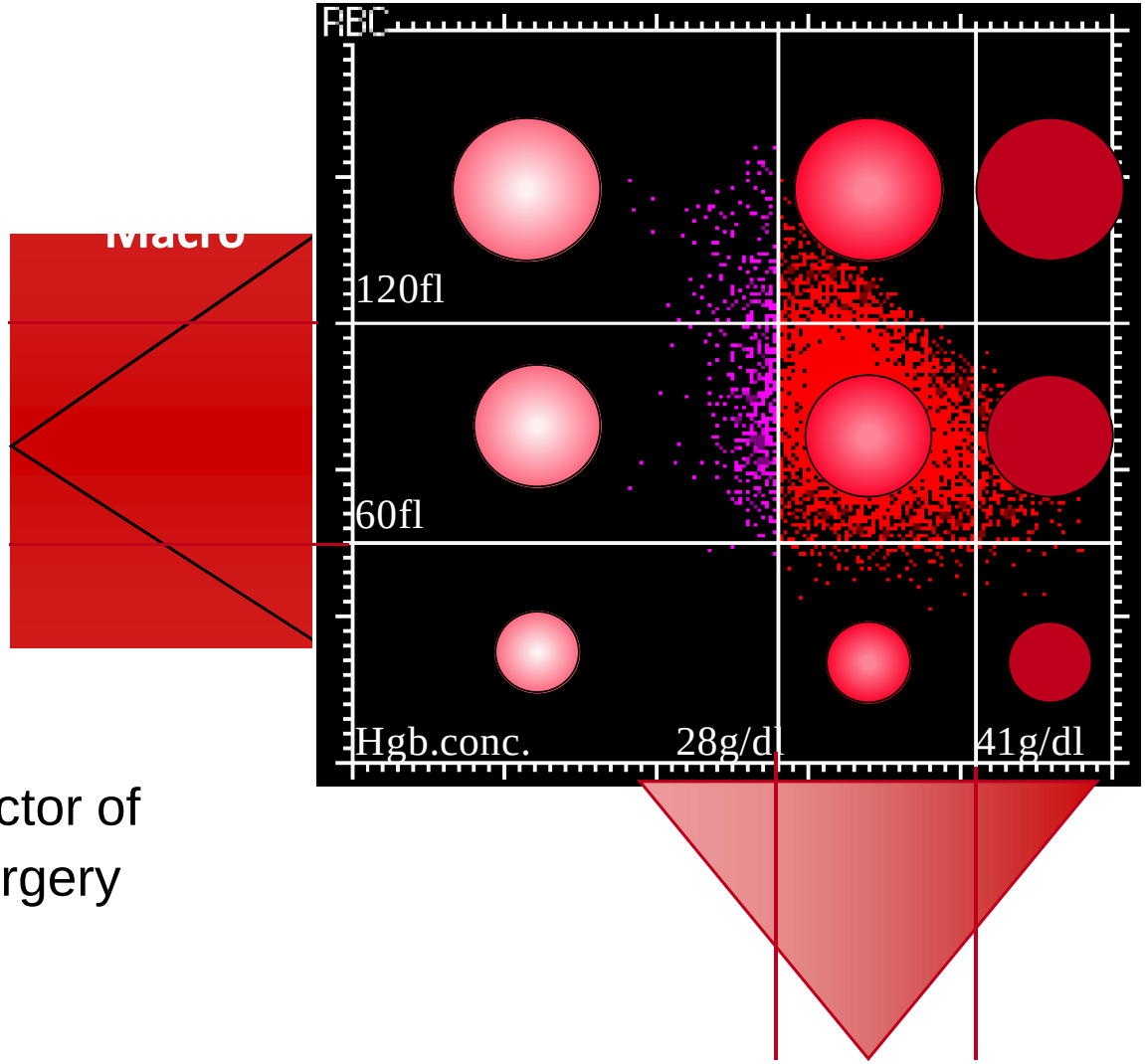
Volume/Hemoglobin Concentration (V/HC) cytogram



1. 60 fL volume marker
2. 120 fL volume marker
3. 28 g/dL HC marker
4. 41 g/dL HC marker

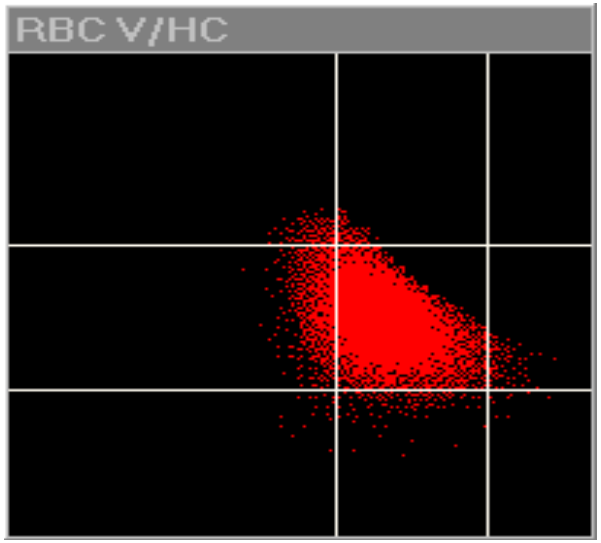
Red Cell Morphology Information ADVIA

- %MICRO
- %MACRO
- %HYPO
- %HYPER

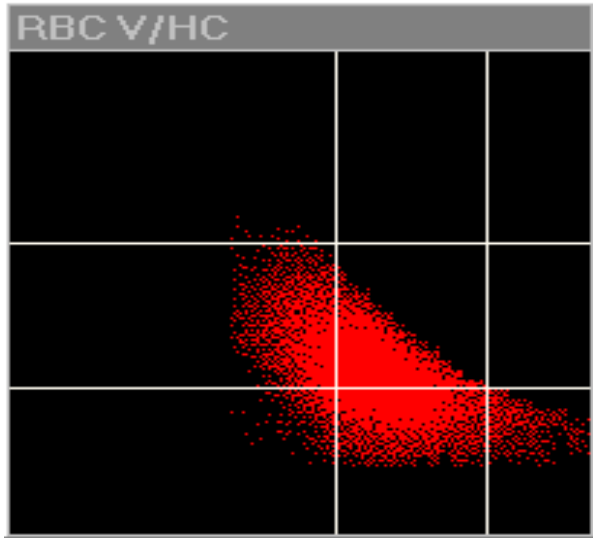


- Aids Thalassaemia diagnosis
- Detects spherocytosis
- % hypochromic cells as predictor of survival in ITU and Gastric surgery

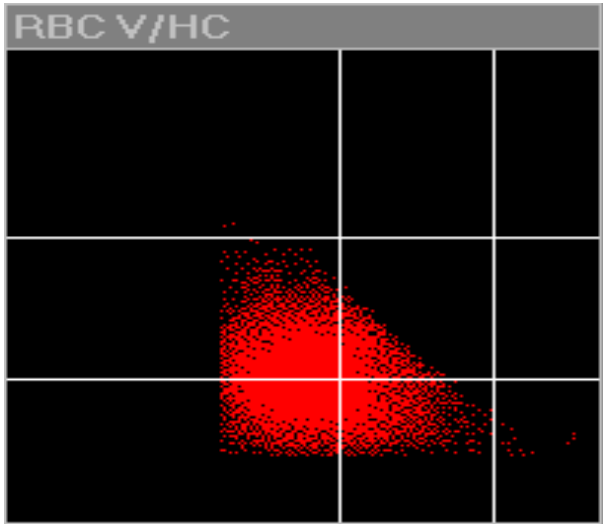
RBC Cytogram: Visual Analysis (ADVIA)



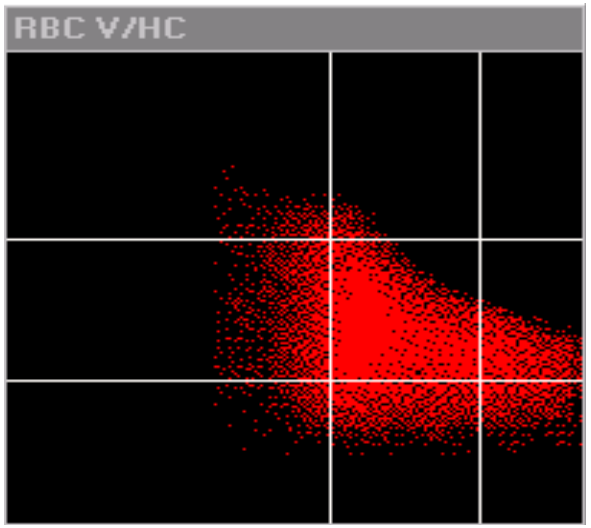
Normal



β-Thalassemia Trait

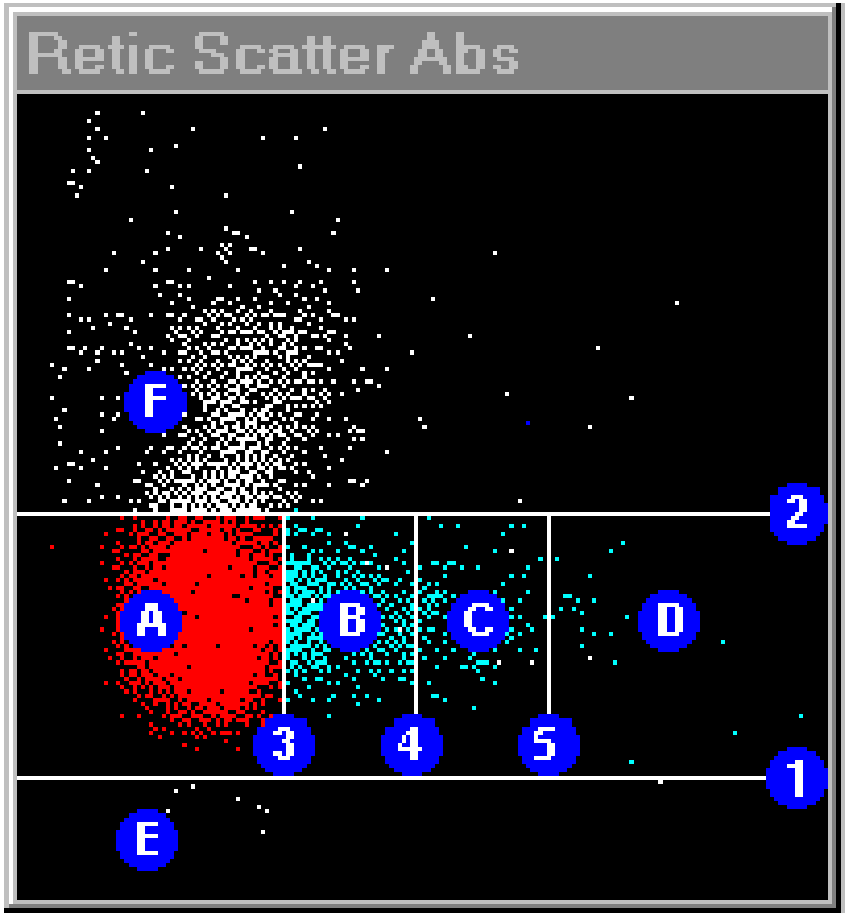


Iron Deficiency Anemia (IDA)

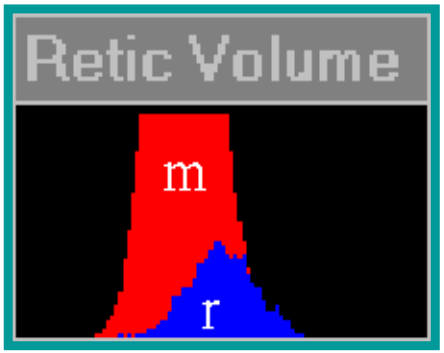


Sickle Cell Anemia

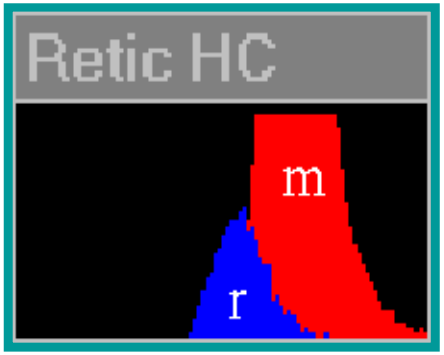
Reticulocyte Parameters (ADVIA)



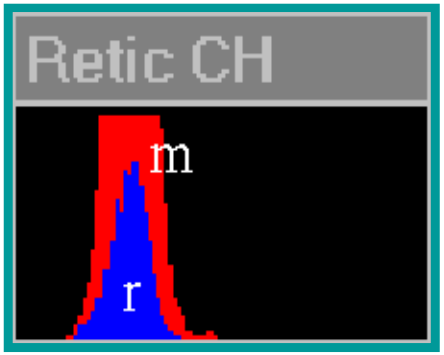
→ IRF-H
→ IRF-M+H



The RETIC Volume histogram
→ **MCVr**

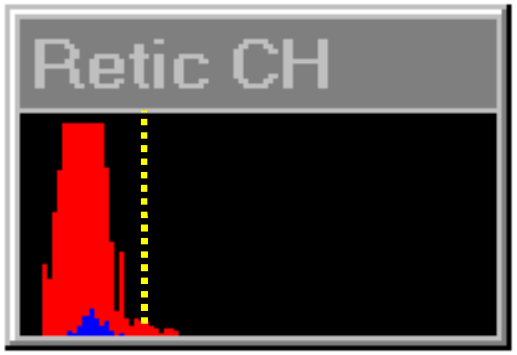


The RETIC hemoglobin concentration (RETIC HC)
→**CHCMr**

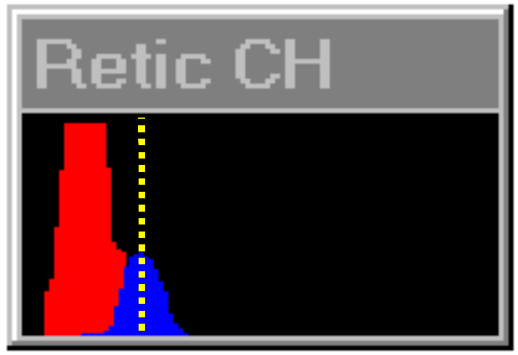


The RETIC cellular hemoglobin (RETIC CH)
→ **CHr**

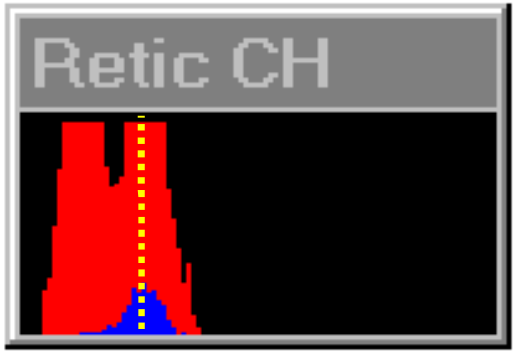
Efficacy of Iron i.v. administration in Iron Deficiency (CHr, ADVIA)



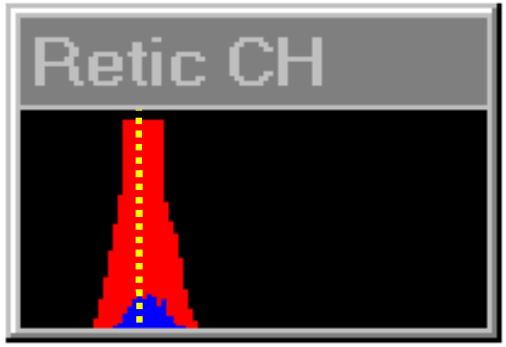
At Diagnosis



After 4 days



After 2 weeks

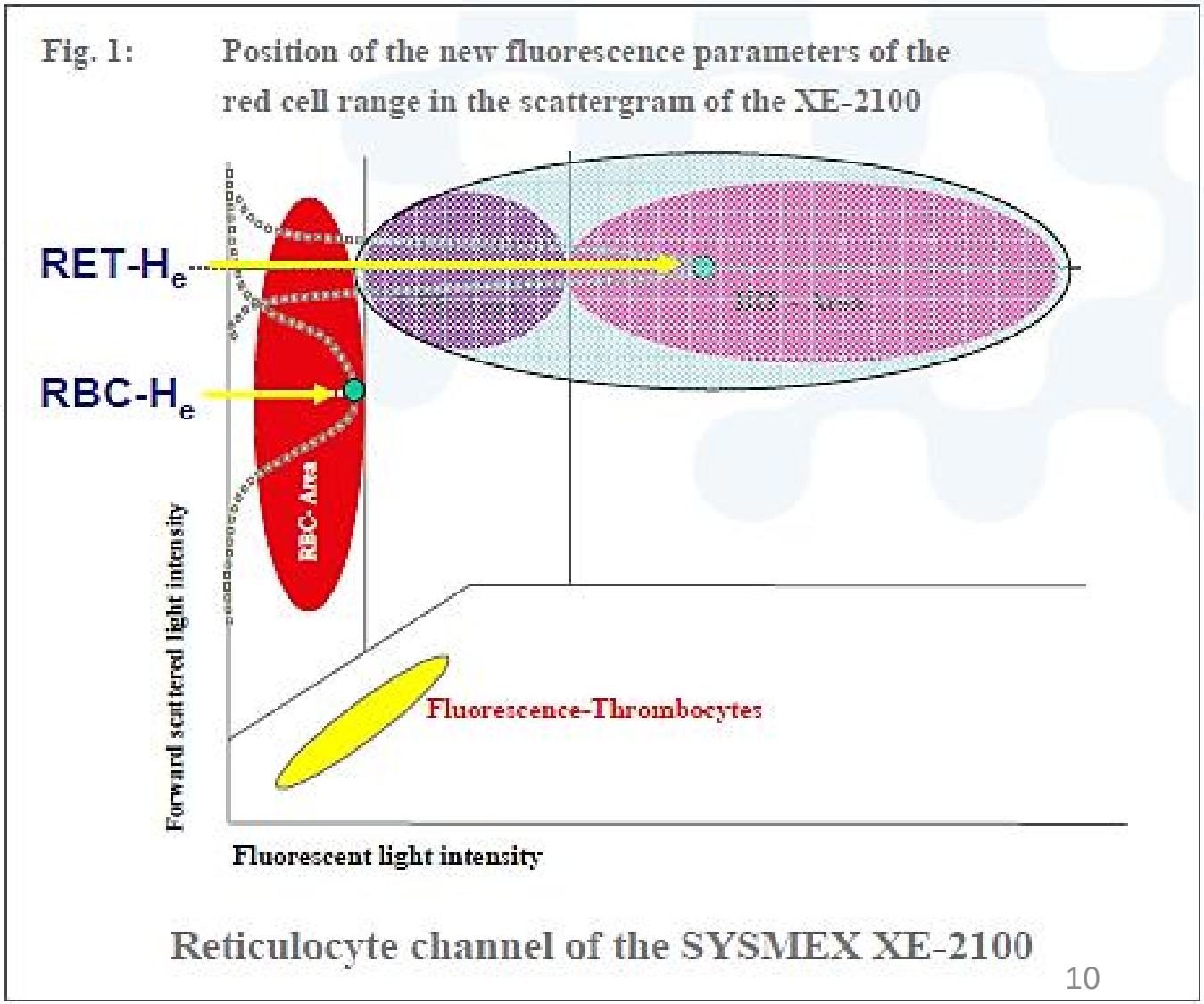


After 1 month

New parameters on XE-2100 (Sysmex)

RBC-He: calculated from RBC-Y; Hb equivalent in mature RBCs (pg)
RET-He: calculated from RET-Y; reticulocyte Hb equivalent (pg)

% Hypo He (Hb content <17 pg)
% Hyper He (Hb content >49 pg**)**



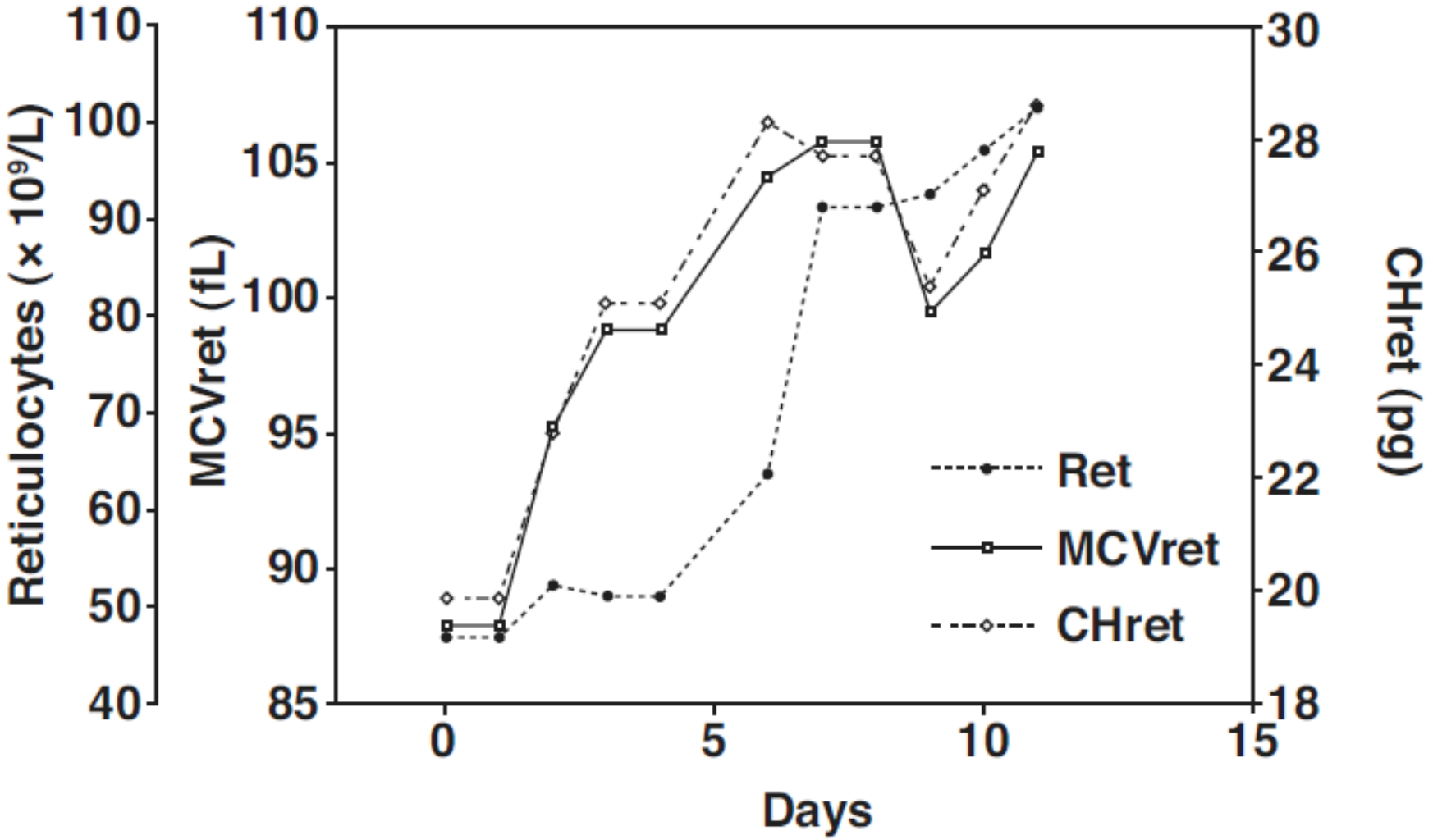
CHr (ADVIA, Siemens) and RET-He (XE-2100, Sysmex)

Hb content of the freshly produced red blood cells Ÿ real-time information on iron availability for the erythropoiesis.

Indication

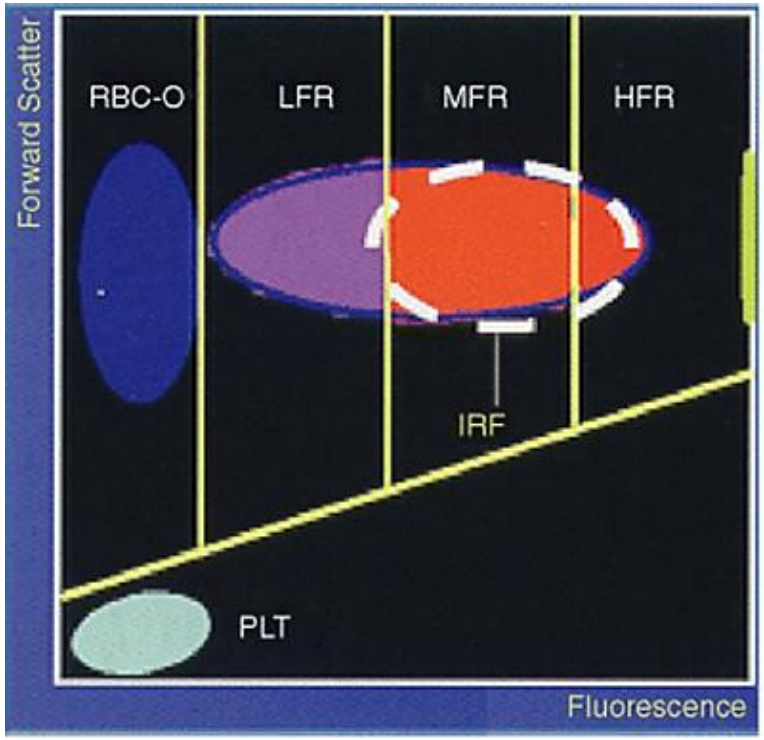
- Differentiate iron deficiency anemia (IDA) and anemia of chronic disease (ACD)Ÿ Differentiate **actual iron deficiency** and **đfunctional iron deficiencyô**(disturbance of iron mobilization)
- Detect latent Fe deficiency
- Monitoring BM response to Epo therapy and iron substitution
  - Recommended by the European Best Practice guidelines for the management of anemia in patients with CRF
  - Good correlation with sTfR
  - Good correlation with BM iron staining

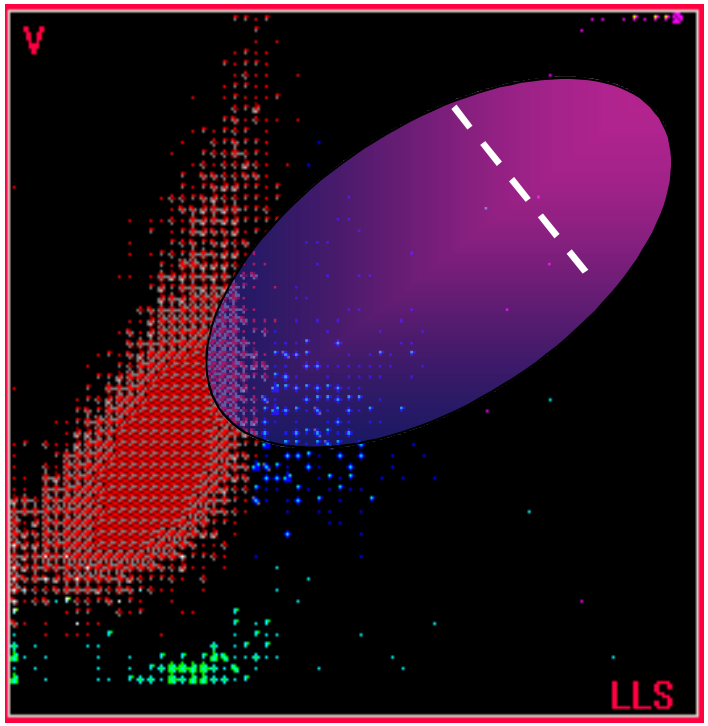
A case of iron deficiency treated with i.v. iron: early and parallel response of MCVr and CHr



Am J Clin Pathol 2008;130:104-116

Immature reticulocyte fractions
Scattergram by Sysmex and Beckman Coulter





+ HLR = High Lightscatter Retics

- Early and sensi□ve index of erythropoiesis, predic□on of ↑CD34+ for best □me collec□on
- BM regeneration (transplantation or chemotherapy)
- DD anemias with ↑ erythropoiesis (AIHA, blood loss) vs anemias with ↓ MB ac□vity (CKD) vs dyserythropoiesis (acute inf, MDS)
- Monitoring efficacy of therapy in nutr.anemias

New parameters on LH750 and DxH 800 (Beckman Coulter)

- IRF (Immature Retic Fraction)
- RDWR
- LHD% (Low Hemoglobin Density %)

$$LHD[\%] = 100 \cdot \sqrt{(1 - [1/e^{1,8 \cdot (30 - MCHC)}])}$$

- MAF (Microcytic Anemia Factor)

$$MAF = (Hb \times MCV) / 100$$

- RSf (Red cell Size factor)

$$RSf = \sqrt{(MRV \times MCV}$$

- MSCV (Mean Sphered Corpuscular Volume)
- UGC% (Unghosted red Cells)

Comparative studies between different instrument parameters

	LHD%	Ret-He	RBC-He	RSf or MAF
% Hypo % Hypo-He	Urrechaga 2009, 2010 (Iron status, IDA, CKD, ACD, Thalas)		David 2005; Brugnara 2006 (IDA and CKD adult and pediatric patients)	
CHr		Thomas 2005; David 2005; Brugnara 2006 (IDA and CKD adult and pediatric patients)		Urrechaga 2008, 2010 (restricted erythopoiesis (IDA and beta thal))
RSf and LHD%		Osta, 2012 (IDA pediatric patients)		

Comparative studies between different instrument parameters

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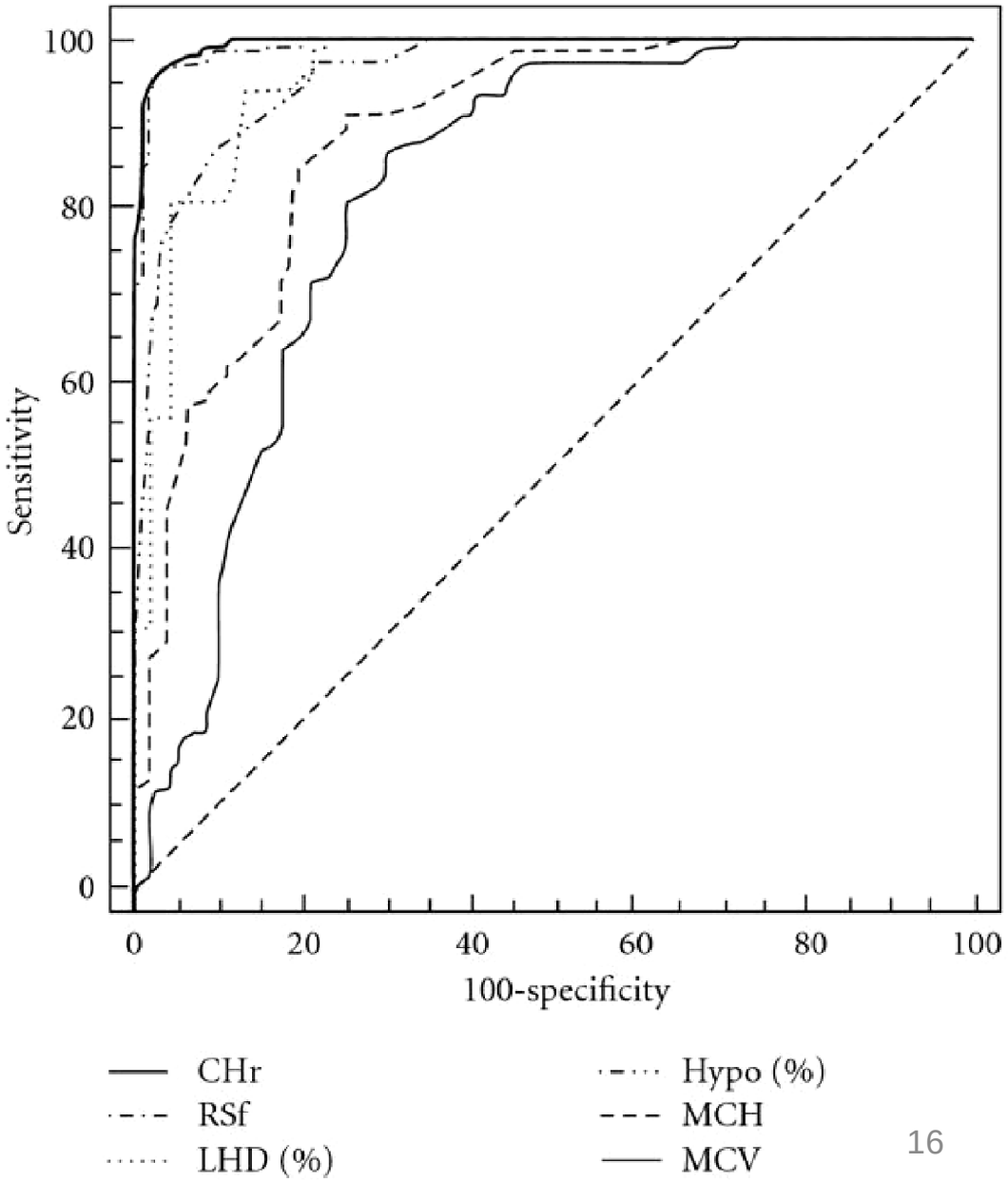
Anemia (2010), Article ID 625919

Erythrocyte and Reticulocyte Indices on the LH 750 as Potential Markers of Functional Iron Deficiency

Eloísa Urrechaga,[1] Luís Borque,[2] and Jesús F. Escanero[2]

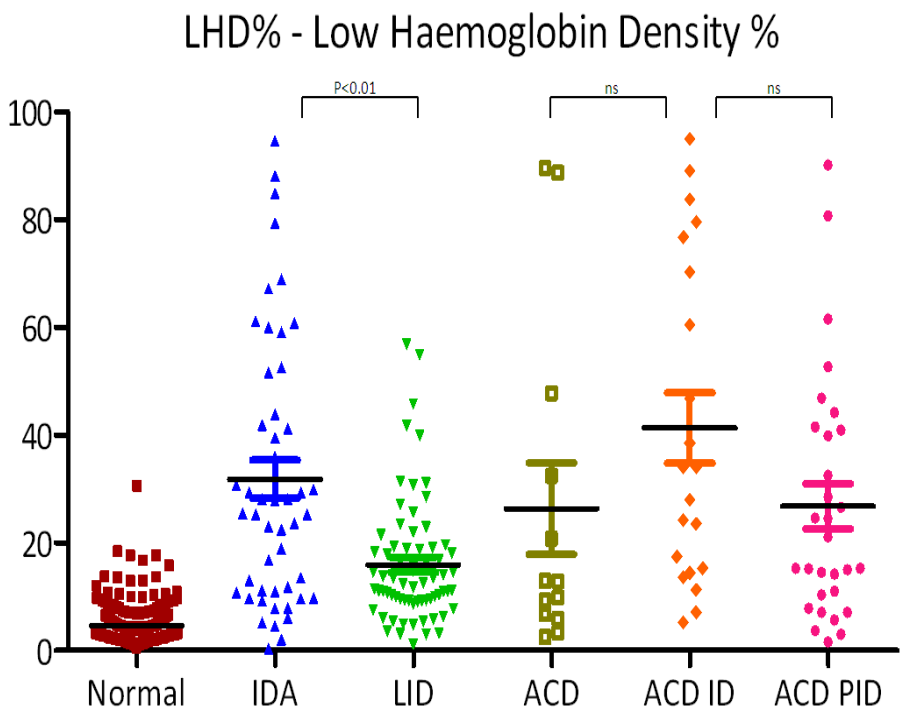
120 healthy ind
132 IDA patients
71 CKD
58 ACD

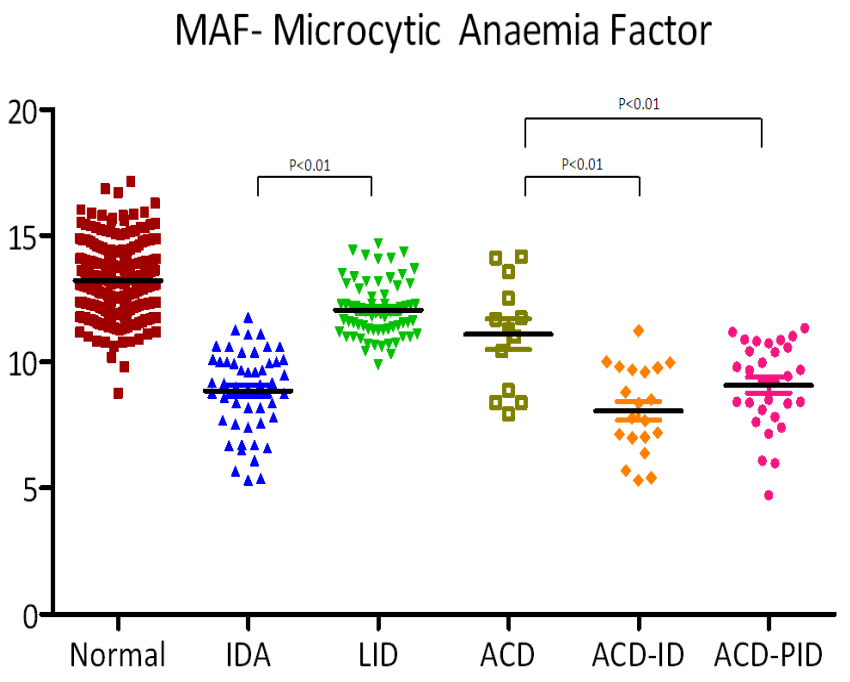
Iron deficiency status defined CHr<28pg



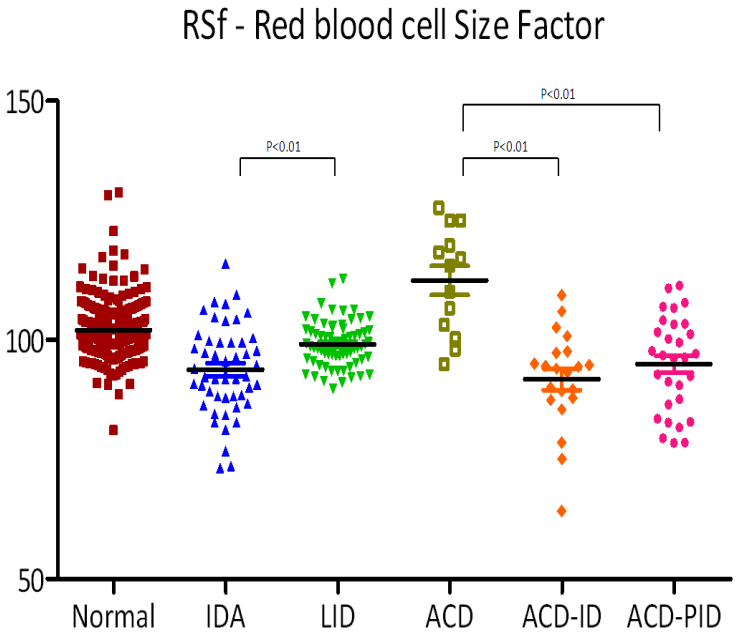
- Iron deficiency with or without anemia
- Anemia of chronic inflammatory diseases
  - our experience

IDA, LID, ACD: our experience



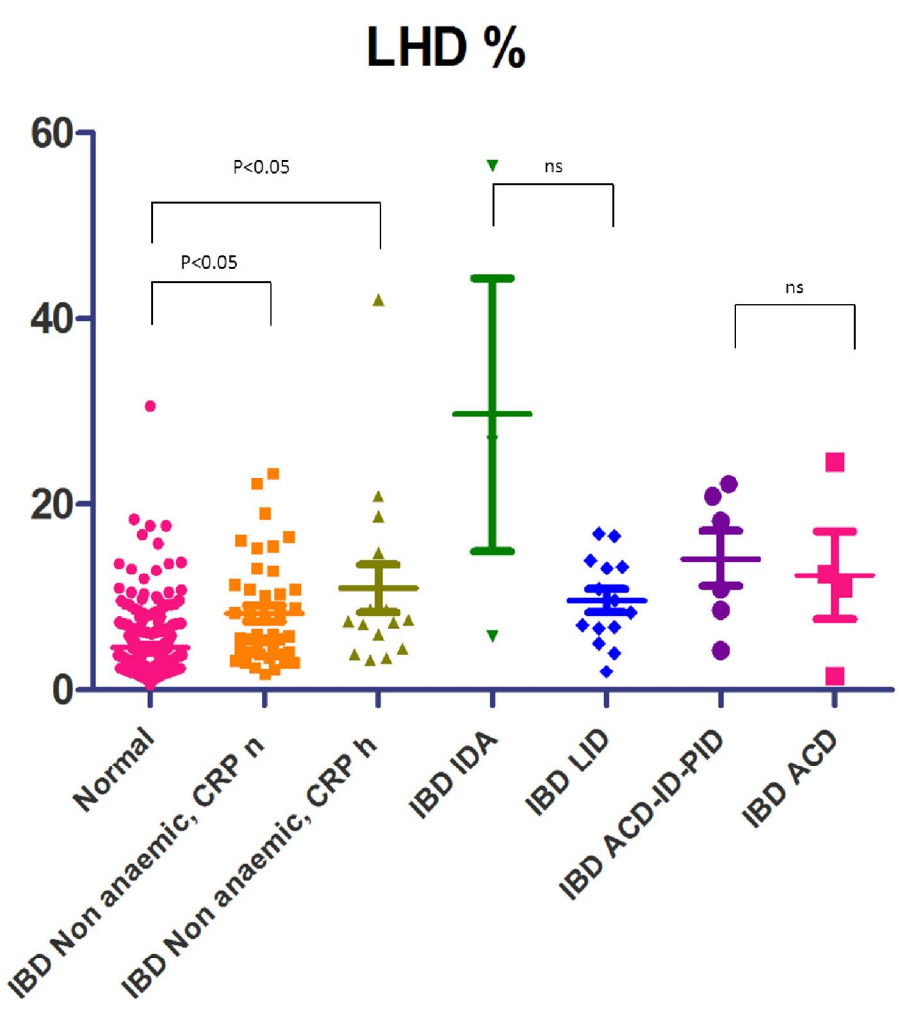


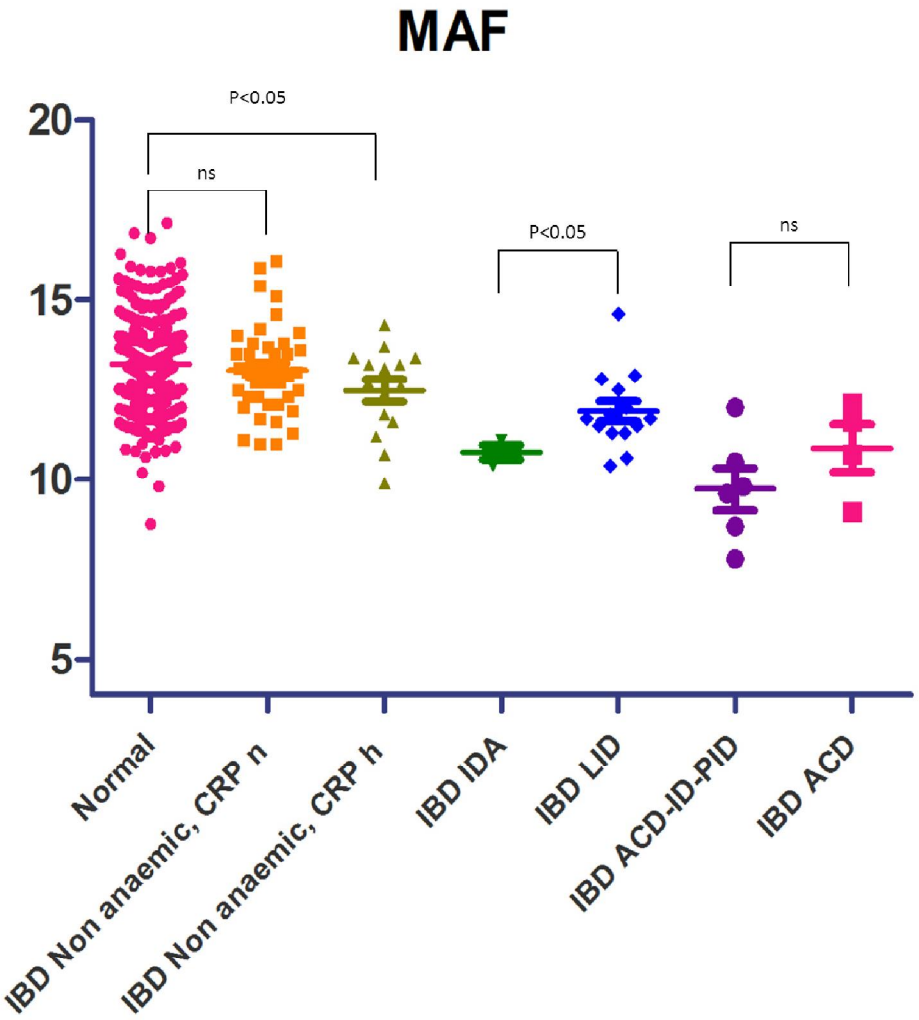
		IDA	LID	ACD-ID	ACD-PID	ACD
Hb (g/dL)	*Female*	<12	12ÒHbÒ15.5	<12	<12	<12
	Male	<13	13ÒHbÒ17	<13	<13	<13
FET (µg/L)		<30	<30	<30	30<FET<100	>100
CRP (mg/L)		<10	<10	>10	>10	>10



LHD%, MAF and RSf could be used as screening tests of latent iron deficiency

LHD% and MAF in inflammatory bowel disease (IBD) patients





MAF could be used in order to diagnose iron depletion in patients with anaemia of chronic disease

- Hereditary spherocytosis
  - our experience

Hereditary spherocytosis (HS)

- HS screening guidelines (Bolton-Maggs *et al*, 2011)
  - family history
  - typical clinical features
  - simple laboratory investigations:
    - spherocytes, ŷ MCHC, ŷ retic
  - reduced area-to-volume ratio, increased osmotic fragility
    - osmotic fragility test, glycerol lysis test, acidified glycerol lysis test, Pink test
    - Cryohaemolysis and eosine 5-maleimide (EMA) binding test: related to red cell membrane protein defects
- Associate two screening tests: ŷnegative and positive predictive values of the tests, e.g. the EMA binding test and the acidified glycerol lysis test (Bianchi *et al*, 2012)

Automated reticulocyte parameters and hereditary spherocytosis (HS)

- **Ret/IRF**, ŷreticulocytes without an equally ŷIRF: useful in HS diagnosis (Mullier *et al*, 2011)

- Beckman Coulter instruments provide the mean sphered corpuscular volume (**MSCV**):
  - whole RBC population volume analysed under hypo-osmotic conditions of the ghosting solution used to leak out Hb before the reticulum definition and to make the red cells swell

  - if **MSCV<MCV** (GEN.S. Coulter), HS could be suggested with a sensitivity of 100% (Chiron *et al*, 1999)
  - MSCV (LH 750): **delta (MCV-MSCV)** >9.6 fl, HS was suspected and DD with AIHA by anti-globulin test was proposed (Broseus *et al*, 2010)

Automated reticulocyte parameters and hereditary spherocytosis (HS): our experience

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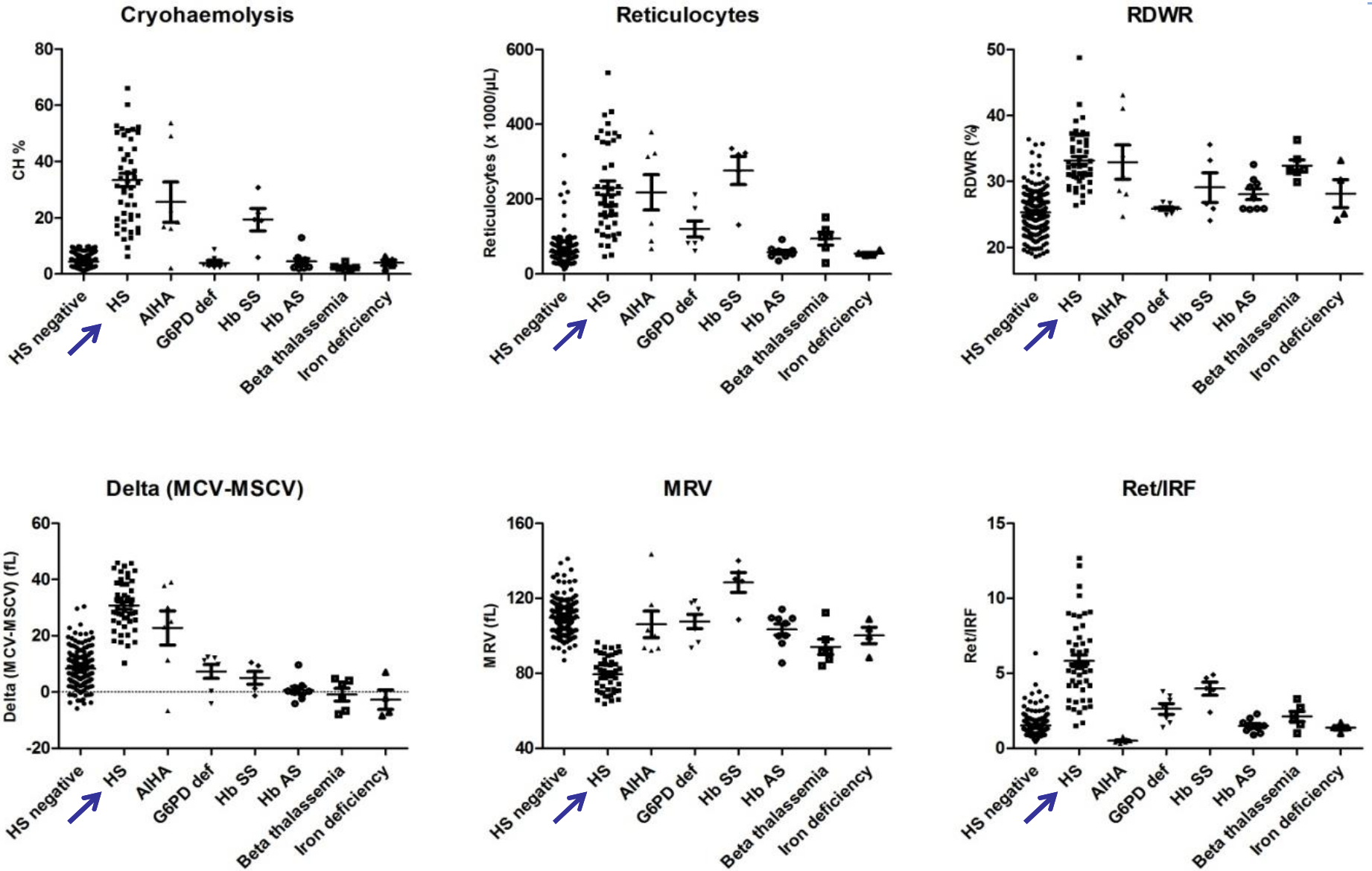
- MSCV, MRV, RDWR and IRF
- Reference values for our population
- Diagnostic performances for HS compared to the cryohaemolysis test
- Efficiency to differentiate HS from other conditions that affect erythropoiesis
- New screening algorithm for HS

HS and other conditions affecting the level of erythropoiesis



Comparison of screening tests results in hereditary spherocytosis patients (HS, n=48), controls (n=213, 82 healthy subjects and 131 cryohaemolysis negative without anaemia) and in patients with anaemia of different origins: auto-immune haemolytic anaemia (AIHA, n=7), G6PD deficient patients (n=7), Hb SS (n=5), Hb AS (n=9), beta-thalassemia patients (n=6), and iron deficiency (n=4)

Automated reticulocyte parameters and hereditary spherocytosis (HS): our experience

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- **MSCV**: in agreement with the published data: the cut-off of delta (MCV-MSCV) >10.4 fL (100% sensitivity) is close to the one of Broséus (9.6)
- **MRV** performances for HS for the first time the
- Statistical differences:
  - HS and the control group (**all parameters**)
  - HS and each of the other pathologies (**all parameters except RDWR**)
  - HS and AIHA (**Ret/IRF ratio, MRV, MSCV, and delta(MCV-MSCV)** contrary to CH%, Ret or RDWR

New screening algorithm:
if delta (MCV-MSCV) > 10.4 and/or MRV < 96.7, the cryohaemolysis test is performed, and if the cryohaemolysis test result is >10%, the confirmatory SDS-PAGE is made.

CONCLUSIONS

- Clinical settings
  - Classification of anemias
  - Early identification of marrow regeneration /engraftment
  - Monitoring the efficacy of therapy in anemia /Epo abuse in sports
  - Resticted erythropoiesis(iron deficiency, beta thalassemia, ACD with/without iron depletion), LID
  - Hereditary spherocytosis
- Reporting of new hematology parameters to clinicians
  - IRF
  - MRV
  - Cell Hb content: %Hypo, %Hyper, %Micro, %Macro, CHr, Ret He, LHD%, MAF
- Reference ranges reporting
  - Method-dependent
- Diagnostic algorithms
  - other lab tests automatically generated
  - comment to the clinician on the lab report