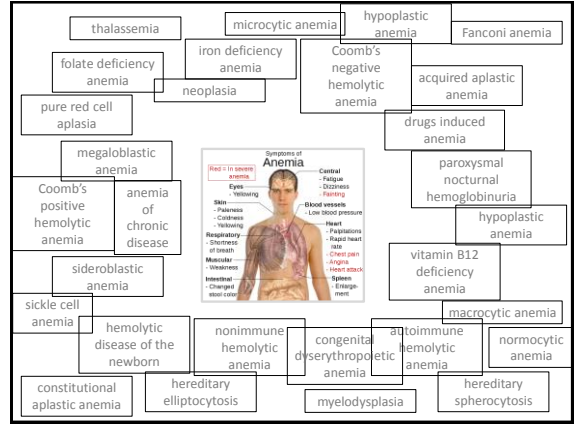


## Anemia ... a challenging diagnostic workout

N. Boeckx, MD, PhD  
UZ Leuven  
13-03-2012



## Definition of anemia

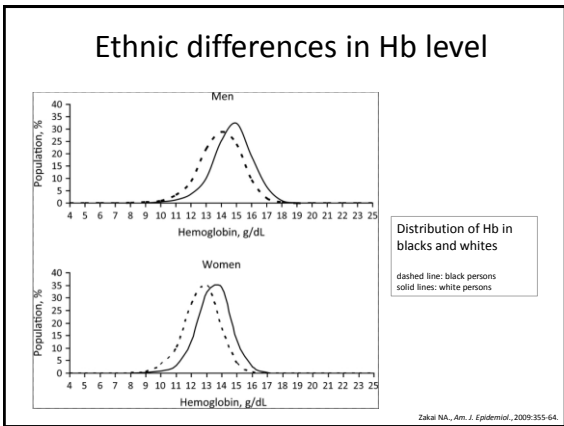
a reduction in one or more of the major RBC measurements

- hemoglobin concentration
- hematocrit
- RBC count

## WHO's hemoglobin thresholds used to define anemia

Age or gender group	Hb threshold (g/dl)	Hb threshold (mmol/l) (1 g/dL = 0.6206 mmol/L)
Children (0.5–5.0 yrs)	11.0	6.8
Children (5–12 yrs)	11.5	7.1
Teens (12–15 yrs)	12.0	7.4
Women, non-pregnant (>15yrs)	12.0	7.4
Women, pregnant	11.0	6.8
Men (>15yrs)	13.0	8.1

World Health Organization (2008). [Worldwide prevalence of anaemia 1995-2005](#). Geneva: World Health Organization. [ISBN 928944196602](#)



## Age-specific normative red blood cell values

Age	Hemoglobin (g per dL)		Hematocrit (%)	
	Mean	SD	Mean	SD
26 to 30 weeks* gestation	13.4	11.0	41.5	34.9
28 weeks* gestation	14.5	NA	45	NA
32 weeks* gestation	15.0	NA	47	NA
Full term (cord sample)	16.5	13.5	51	42
1 to 3 days	18.5	14.5	56	45
2 weeks	16.6	13.4	53	41
1 month	13.9	10.7	44	33
2 months	11.2	9.4	35	28
6 months	12.6	11.1	36	31
6 months to 2 years	12.0	10.5	36	33
2 to 5 years	12.5	11.5	37	34
6 to 12 years	13.5	11.5	40	35
12 to 15 years (male)	14.5	13.0	43	36
12 to 15 years (female)	14.0	12.0	41	37
Adult (male)	15.5	13.5	47	41
Adult (female)	14.0	12.0	41	36

NA = not available. SD = standard deviation.

Janus J, et al. *Am Fam Physician* 2010;81:1462-71.

## Causes of anemia

- **Kinetic approach**
  - decreased RBC production
  - increased RBC destruction
  - blood loss
- **Morphologic approach (RBC size)**
  - macrocytic anemia
  - microcytic anemia
  - normocytic anemia

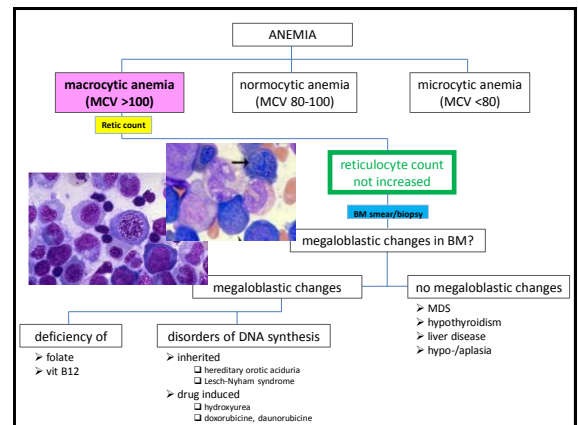
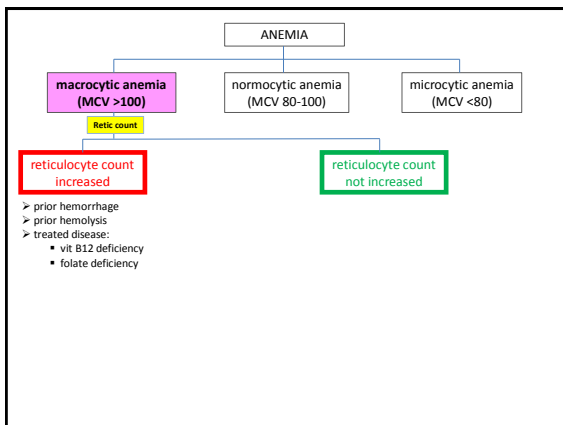
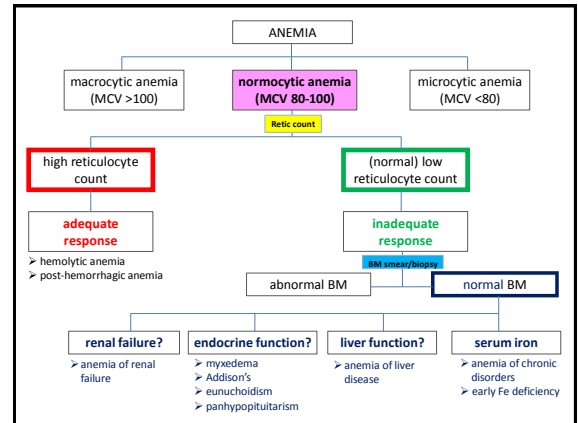
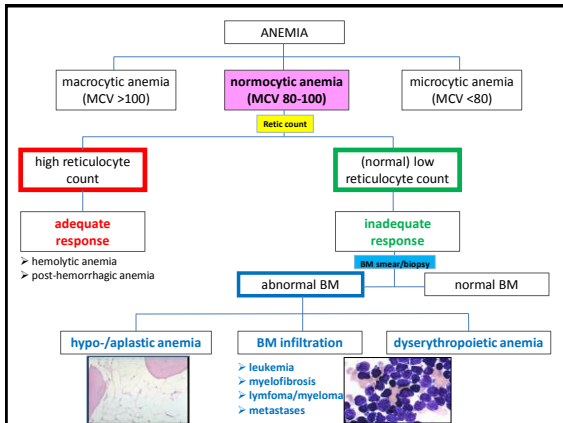
## Red blood cell indices

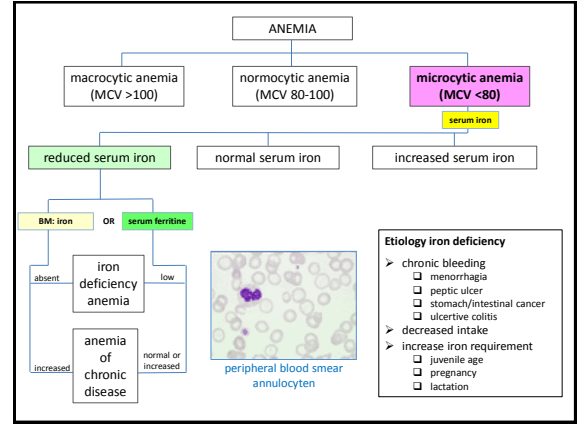
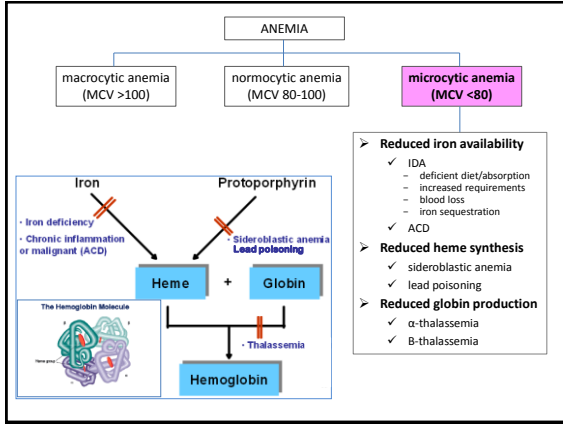
### 'old' parameters

- RBC
- Hb
- Hct
- MCV
- MCH
- MCHC
- RDW

### 'new' parameters

- Reticulocytes (%)
- Reticulocytes ( $10^9/L$ )
- Immature reticulocyte fraction
- Reticulocyte hemoglobin content
- Reticulocyte volume

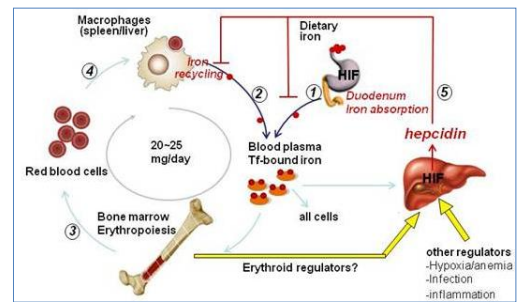




## Regulation of iron balance

- Body iron content: 3-4 g
  - most body iron (65-70%) is present in **hemoglobin** in circulating red cells
  - storage (**ferritin** and **hemosiderin**)
  - transferrin transports iron into the cells
- Iron homeostasis is strictly regulated:
  - daily intake:** absorption via duodenum (10% of daily iron is absorbed : 1-3 mg daily)  $Fe^{2+}$  better absorbed than  $Fe^{3+}$
  - daily small loss** of iron via urine, faeces, skin and nails and in menstruating females as blood (1-2 mg daily)

## Iron homeostasis



## Iron deficiency stages

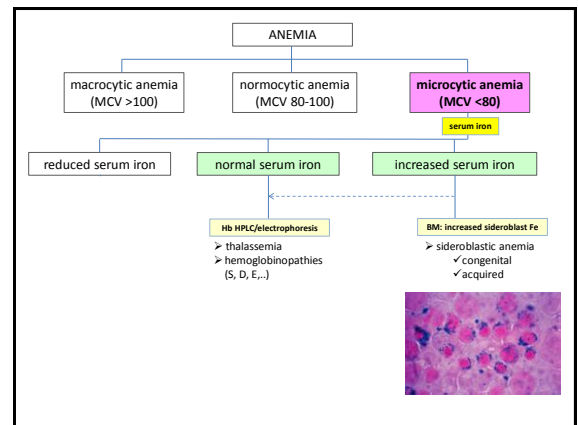
**Prelatent:** reduction in iron stores WITHOUT reduced serum iron levels

**Latent:** iron stores are exhausted, but Hb-LEVEL remains NORMAL

**Iron deficiency anemia:** Hb-CONCENTRATION FALLS below lower limit of normal

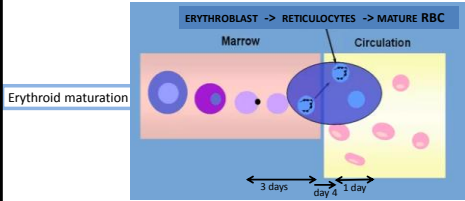
	PRELATENT	LATENT	IDA
Hb	nl	nl	↓
MCV	nl	nl (RDW ↑)	↓
ferritin	↓	↓	↓
marrow iron	↓	absent	absent

Additional markers for IDA: hypochromic normocytic RBC, hypochromic microcytic anemia.



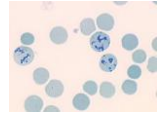
## Reticulocyte

- immature red blood cell
- about 0.5-1.5% of red cells in human body (non-anemic)
- develop and mature in bone marrow
- circulate for about 1 day in blood stream before developing into mature red blood cell



## Reticulocyte counting

- **Microscopic counting** (supravital staining of cytoplasmic ribosomal RNA)
  - ⇒ rather imprecise
    - different procedures
    - staining variations
    - distributional variability of quality blood film
    - inter- and intra observer variations
- **Automated counting** (available in the mid-1990's)
  - ⇒ increasing measurement precision by analyzing a much greater number of cells
  - Elimination of variability of pre-analytic staining, dilution and incubation, variability due to subjective elements
  - ⇒ also measurement of additional parameters (such as the mean reticulocyte volume (MRV) and reticulocyte hemoglobin content (CHR, Ret He),...)



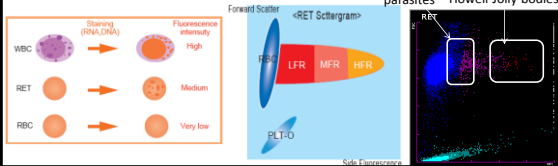
**BLOOD CONCENTRATION OF RETICULOCYTES REPRESENTS A QUANTITATIVE MEASURE OF ERYTHROPOIESIS**

**RETICULOCYTE PARAMETERS PROVIDE REAL-TIME INFORMATION ABOUT THE QUALITY OF THE ERYTHROPOIESIS**

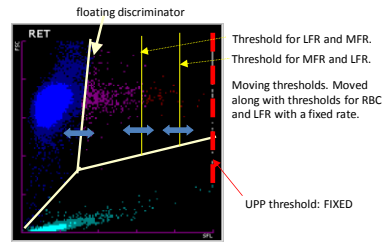
## Retic counting on Sysmex XE-5000

### RET-channel

- ✓ dye (polymethine)
- ✓ penetration of the cell membrane
- ✓ staining of RNA (retics) and DNA/RNA (nucleated cells)
- ✓ measurement of forward scatter and side fluorescence
- ✓ separation of retics from mature RBC and nucleated cells
- ✓ retics divided in 3 ranges: LFR, MFR, HFR
- ✓ MFR + HFR = IRF
- ✓ NOTE: PLT-O also determined
- ✓ no interference from NRBC, WBC



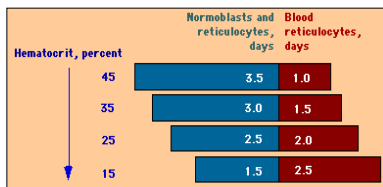
## Retic counting on Sysmex XE-5000



To be useful reticulocyte count must be adjusted for patient's hematocrit.

When hematocrit is low(er), reticulocytes are released earlier from the marrow so one can adjust for this phenomenon.

⇒ Reticulocyte correction



## Reticulocyte Correction

➤ Correction for the degree of anemia  
**CORRECTED RETICULOCYTE COUNT**

➤ Correction both for Hct and maturation time  
**RETICULOCYTE PRODUCTION INDEX (RPI)**

Correction for the longer life span of prematurely released reticulocytes in the blood (a phenomenon of increased RBC production).

➤ **Absolute reticulocyte count** = reticulocyte count x # RBC

## Calculations

- CORRECTED RETICULOCYTE COUNT**  
patients reticulocyte count (%) x (patients Hct / normal Hct (=45))
- RETICULOCYTE PRODUCTION INDEX (RPI)**  
RPI: corrected reticulocyte count / maturation time

Hct (%)	retic survival (days) = maturation correction
36-45	1.0
26-35	1.5
16-25	2.0
<15	2.5

EXAMPLE:  
patient has reticulocyte count of 5%, Hb of 7.5 g/dL and Hct of 25%  
RPI = (5x(25/45)) / 2 = 1.38

## Reticulocyte Production Index

Normal RPI = 1 (for non-anemic patients)  
RPI ≥2 suggests an effective erythropoiesis response  
RPI <2 suggests an ineffective response

Hypoproliferative	Hyperproliferative
Iron deficiency anemia	Hemolytic disease
B12/folate deficiency	Hemoglobinopathy (including thalassemia)
Anemia of chronic disease	Hemorrhage
Sideroblastic anemia	
Aplastic anemia	

reticulocyte maturation parameters, such as **immature reticulocyte fraction (IRF)**, provides the same clinical significance as the RPI

Most commonly used automated reticulocyte analyzers + parameters

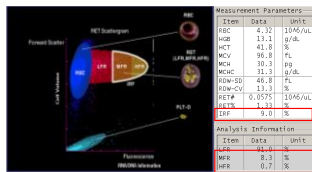
Company	Instrument	Method	Dye	IRF	MW/MCV	RDW	RDW-CV	RDW-S	RDW-SD	RDW-UI	RDW-FL	RDW-PL	RDW-UL	RDW-LL	RDW-UL	RDW-LL	RDW-UL	RDW-LL	RDW-UL	RDW-LL			
Abbott	CELL DYN Sapphire	Fluorescence detection	Cyanine dye	x																			
	Cell Dyn 4000		CD45/3D	x																			
	Cell Dyn 3700			x																			
Beckman Coulter	UniCell Delt 8000	Absorbance	New methylene blue	x																			
	Coulter LH 700 series			x	x	x	x	x	x	x	x												
	Coulter LH 500			x	x																		
	Coulter Hmx			x	x																		
	Coulter Gen <sup>5</sup> S			x	x	x																	
Horiba	ABX Pentra DX 120	Impedance conductivity technology and fluorescence detection	Thiazole orange	x	x																		

Most commonly used automated reticulocyte analyzers + parameters

Company	Instrument	Method	Dye	IRF	MW/MCV	RDW	RDW-CV	RDW-S	RDW-SD	RDW-UI	RDW-FL	RDW-PL	RDW-UL	RDW-LL	RDW-UL	RDW-LL	RDW-UL	RDW-LL	RDW-UL	RDW-LL	
Siemens	ADVIA 2120	Absorbance and optical light scatter detection	Oxazine 750	x	x																
	ADVIA 120			x	x																
Sysmex	XE5000	Fluorescence and light scattering	Polymethine	x																	
	XE2100			x																	
	SEI500, R series	Fluorescence	Auramine D	x																	

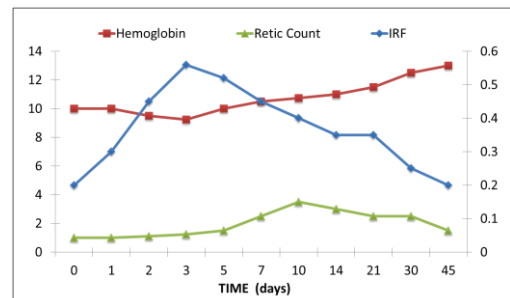
## Immature reticulocyte fraction (IRF)

- Replaces need for "corrected" reticulocyte count
  - Originally called 'reticulocyte maturity index' (RMI)
  - To indicate the less mature reticulocyte fraction
  - Some divide the reticulocytes into 3 distinct populations (eg. Sysmex XE), others into only 2 based on RNA content
- Sysmex : IRF = HFR + MFR (ref. range: 5-21%)



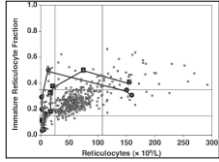
- Reflects rate of erythropoietic activity; early + sensitive index for erythropoiesis

## Changes in RBC parameters (Hb, Retic, IRF) with stimulated erythropoiesis



## Clinical applications of IRF

- ✓ early identification of BM regeneration after chemo (prior to increase # neutrophils and total reticulocyte count)
- ✓ early identification of hematopoietic engraftment (Tx)



Am. J. Clin Path. 2002;117:871-9. Buttarelli M et al.

- ✓ monitor EPO therapy (eg. renal failure)
- ✓ monitoring renal engraftment (EPO production)

## Clinical applications of IRF

- ✓ transfusion assessment
  - low retic + low IRF: no production => transfuse
  - low retic + high IRF: production => do not transfuse
- ✓ distinguishing different types of anemias
  - anemia with increased BM erythropoiesis (eg. hemolytic anemia, blood loss): ↑ retic, ↑ IRF
  - anemia with reduced BM activity (eg. MDS): ↓ to normal retic, ↑ IRF
- monitoring therapeutic efficacy in nutritional anemias (B<sub>12</sub>, folates, iron): ↑ IRF precedes ↑ retic
- diagnosis and monitoring of aplastic anemia

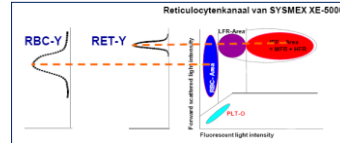
## Patterns of IRF and retic counts in anemia

Clinical conditions	Reticulocyte count	IRF
Aplastic anemia	low	low
Hypoplastic anemia	low	low
BM regeneration	low	high / WNL
Chronic disease	low / WNL	WNL
Iron deficiency	low / WNL	high
Thalassemia	WNL / high	WNL / high
Folate/B12 deficiency	low / WNL	high
MDS	any level	WNL / high
Hemolytic anemia	high	high
Blood loss	WNL / high	high

## Reticulocyte hemoglobine

- Provides hemoglobin content information on immature RBCs (retics)
  - Hb and MCH** ↔ **Ret-He** (Sysmex), cfr. **CHR** < ADVIA Siemens
  - 0-120 days (including all RBC)      4 days
  - Ret-He and Chr correlates in the same distal marrow

- Utilizes fluorescent flow cytometry



forward scatter signal (RET-y) of a stained reticulocyte is highly correlated with its haemoglobin content (MCH): this is used to transform RET-y channel number generated to reticulocyte Hb concentration equivalent expressed in pg

- An estimate of the **recent functional availability of iron** into the erythron

## Clinical utility of Ret-He/RET-Y/CHR

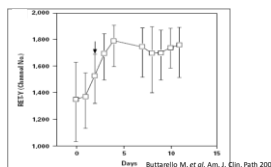
- Diagnosis of iron deficiency (absolute and functional)

**Absolute iron deficiency:** depletion of iron stores and absence of stainable iron in bone marrow  
**Functional iron deficiency:** stored iron is sufficient



- Monitoring response on IV or oral iron substitution

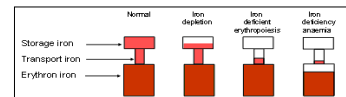
- Hb (>1 m)
- Reticulocytose (5-10 d)
- Ret-He (2-3 d)



## Clinical utility of Ret-He/RET-Y/CHR

- Evaluation of iron status in dialysis patients (planning of adequate r-HuEPO R/ and iron substitution)

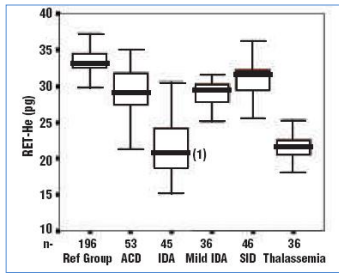
- Iron deficiency in childhood (before anemia)



### Pitfalls

- hemoglobinopathy: Ret-He ↓↓↓, normal Fe-parameters
- megaloblastic anemia: Ret-He normal, despite concomittant Fe-deficiency

### Clinical utility of RET-He in the diagnosis of anemia



ACD: anemia of chronic disease  
IDA: iron deficiency anemia  
SID: storage iron deficiency

Canak C. et al. Haematologica, 2005; 90:1133-4.

### Case 1

- Man 69j
- Klinische info: oedemen, dyspnee, melena, epigastrische pijn
- VG: cardiovasculaire problematiek (fem-pop bypass, angor pectoris, art. hypertensie), sigmoiddiverticulose

		13-02-2012	14-02-2012	
Hemoglobine	g/dL	14.0 - 18.0	6.3	7.4
Hematocriet		0.40 - 0.54	0.210	0.233
RBC	10e12/L	4.50 - 6.00	2.62	2.94
MCV	fL	76.0 - 96.0	80.2	79.3
MCH	pg	27.0 - 32.0	24.0	25.2
MCHC	g/dL	30.0 - 35.0	30.0	31.8
RDW	%	11.7 - 14.5	20.2	18.8
Erythroblast	/100 WBC		1.2	1.4
Erythroblast	10e9/L		0.1	0.1

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Reticulocyten	10e9/L	20 - 100	40	40
IRF	%	5.0 - 21.0	12.2	12.2
Ret-He	pg	30.3 - 35.7	15.6	15.6

### Case 1

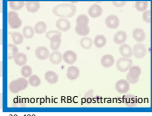
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IRF	%	5.0 - 21.0	12.2	12.2
Ret-He	pg	30.3 - 35.7	15.6	15.6
Iizer	µg/dL	65 - 175	25	25
Transferrine	g/L	2.00 - 3.60	2.97	2.97
Transf sat	%	16 - 45	6	6
Ferritine	µg/L	30 - 400	12	12

### Case 1

- Man 69j
- Klinische info: oedemen, dyspnee, melena, epigastrische pijn
- VG: cardiovasculaire problematiek (fem-pop bypass, angor pectoris, art. hypertensie), sigmoiddiverticulose

		13-02-2012	14-02-2012	15-02-2012	16-02-2012
Hemoglobine	g/dL		7.4	8.7	5.1
Hematocriet		0.233	0.271	0.289	
RBC	10e12/L	2.94	3.41	3.6	
MCV	fL	79.3	80.3		
MCH	pg	25.2	25.3		
MCHC	g/dL	31.8	31.5		
RDW	%	18.8	19.2		
Erythroblast	/100 WBC	1.2	1.4	2.4	
Erythroblast	10e9/L		0.1	0.3	
Reticulocyten	10e9/L		40		
IRF	%		12.2		
Ret-He	pg	15.6	16.9	⇒ ???	
Iizer	µg/dL		35		
Transferrine	g/L		2.97		
Transf sat	%		6		
Ferritine	µg/L		12		



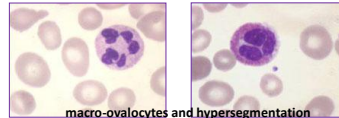
### Case 2

- 58-year female
- extreme alcohol abuse, duodenal ulcer, liver cirrhosis

#### CBC:

- pancytopenia
- Hb 3.7 g/dL ⇒ severe macrocytic anemia
- MCV 141 fL
- reticulocyte count: 10.2 x 10<sup>9</sup>/µL ⇒ ineffective erythropoiesis
- RPI = 0.2

#### blood smear:



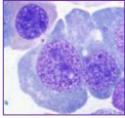
Sysmex Case forum

## Case 2

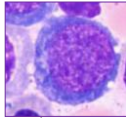
### serum parameters:

- increased LDH (1483 U/L)
  - decreased vitamin B12 (104 pg/mL)
  - decreased folic acid (2,57 ng/mL)
  - CRP normal
  - low normal ferritin (58 ng/mL)
- ➔ **megaloblastic anaemia**  
due to nutritional deficiency, malabsorption of vitamin B12 and folic acid

### bone marrow



Different megaloblasts



Vacuolisation in a proerythroblast

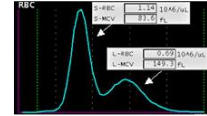
Sysmex Case forum

## Case 2

### Treatment:

#### 1) transfusion of several units of PC

- Hb 7.0 g/dL
- RBC histogram: 2 distinct RBC populations
  - ☐ transfused RBC with a normal MCV (83.6 fL; RBC 1.14 x10<sup>6</sup>/μL)
  - ☐ residual macrocytic cells (149.3 fL; RBC 0.69 x10<sup>6</sup>/μL)



dimorph RBC population

#### 2) Cobalamin (vitamin B12) IV

- cumulative Hb (day 1: Hb 3,7 => day 18 : Hb 8,5)
  - increase of reticulocytes (225.900/μL)
  - increase of IRF (27%)
  - RPI = 2.4
  - normalisation platelets and leucocytes
- ➔ **effective erythropoiesis**

Sysmex Case forum

## Laboratory evaluation

- **initial testing**
  - CBC with differential, including RBC indices
  - reticulocyte count
  - peripheral blood smear
  - serial Hct or Hb
- **iron deficiency / nutritional deficiency**
  - iron studies / folate, vitamin B12
- **hemolysis**
  - serum LDH, indirect bilirubin, haptoglobin, coombs
- **bone marrow examination**
- **others-directed by clinical indication**
  - hemoglobin electrophoresis