
# ERN-EuroBloodNet Webinars for Patients:
# Congenital Dyserythropoietic Anemia I and Congenital Dyserythropoietic Anemia II
## Diagnostic aspects

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ERN-EuroBloodNet subnetwork RBC disorders
Milan – Italy
25 May 2021

Co-funded by
the Health Programme
of the European Union

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for rare or low prevalence complex diseases
Network Hematological Diseases (ERN EuroBloodNet)

# Conflicts of interest

**Paola Bianchi BSc, PhD**

**No confilct of interest  to declare**

# Webinar rules

- **30-35min presentation (30 slides max)  + 15 min Q&A session**
- **Microphones will be muted by host to avoid back noise**
- **Please, stop your video to improve internet conexion**
- **Send your questions during the presentation through the chat**

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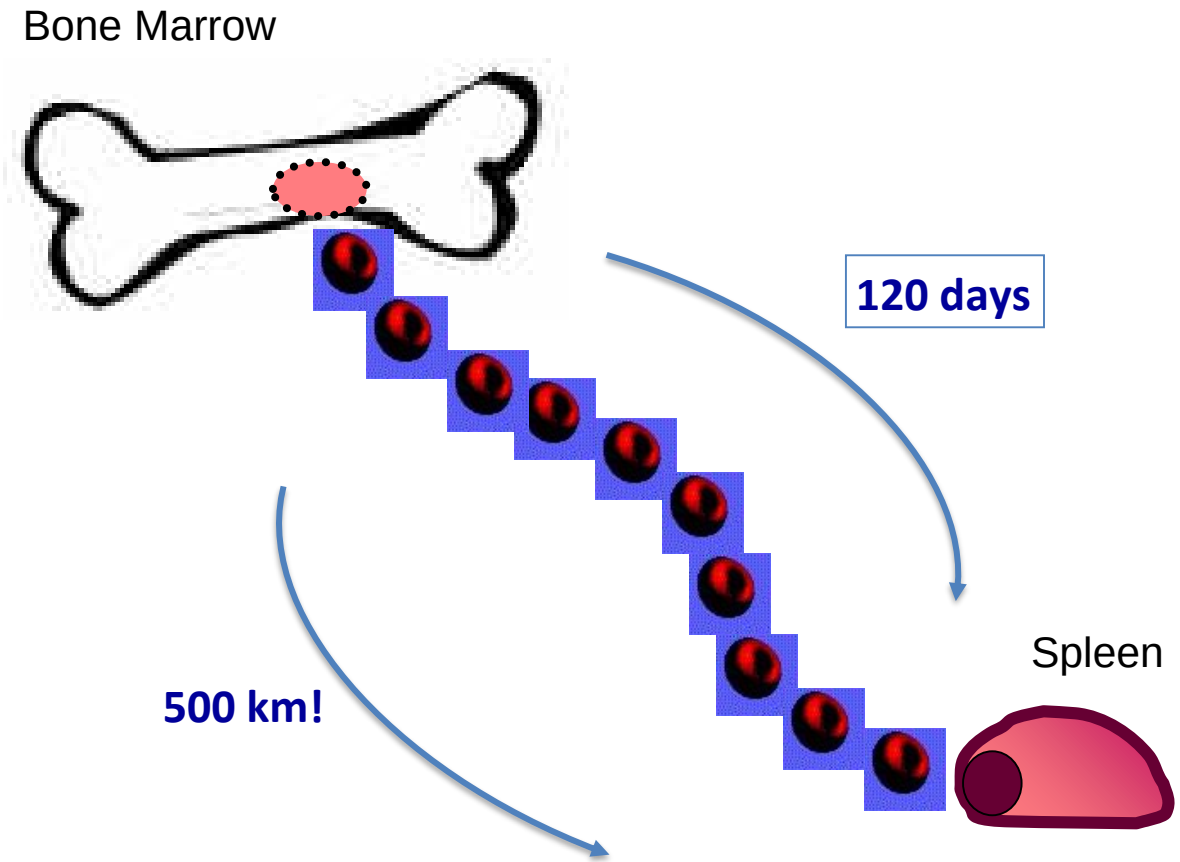
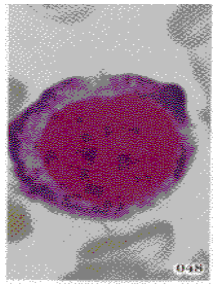
# Learning objectives

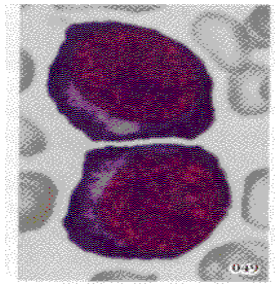
1. When to suspect  a CDAs
2. Typical laboratory features in CDAI and CDAII
3. Molecular basis of CDAI and CDAII



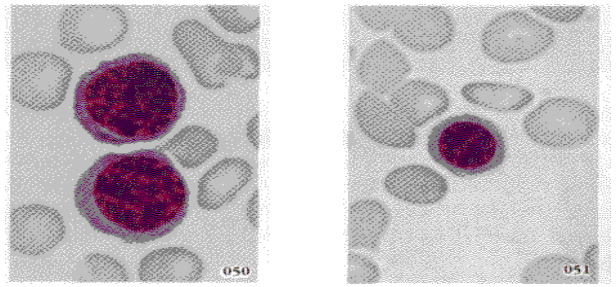

Proerythroblasts



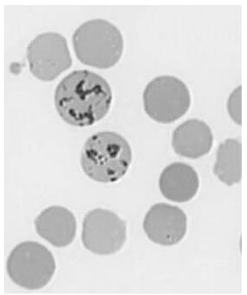
Polychromatic erythroblasts



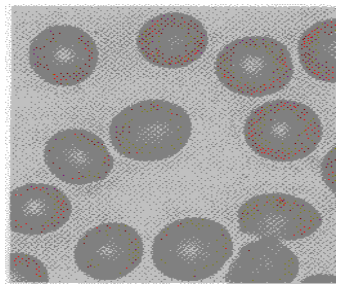
Orthochromatic erythroblasts

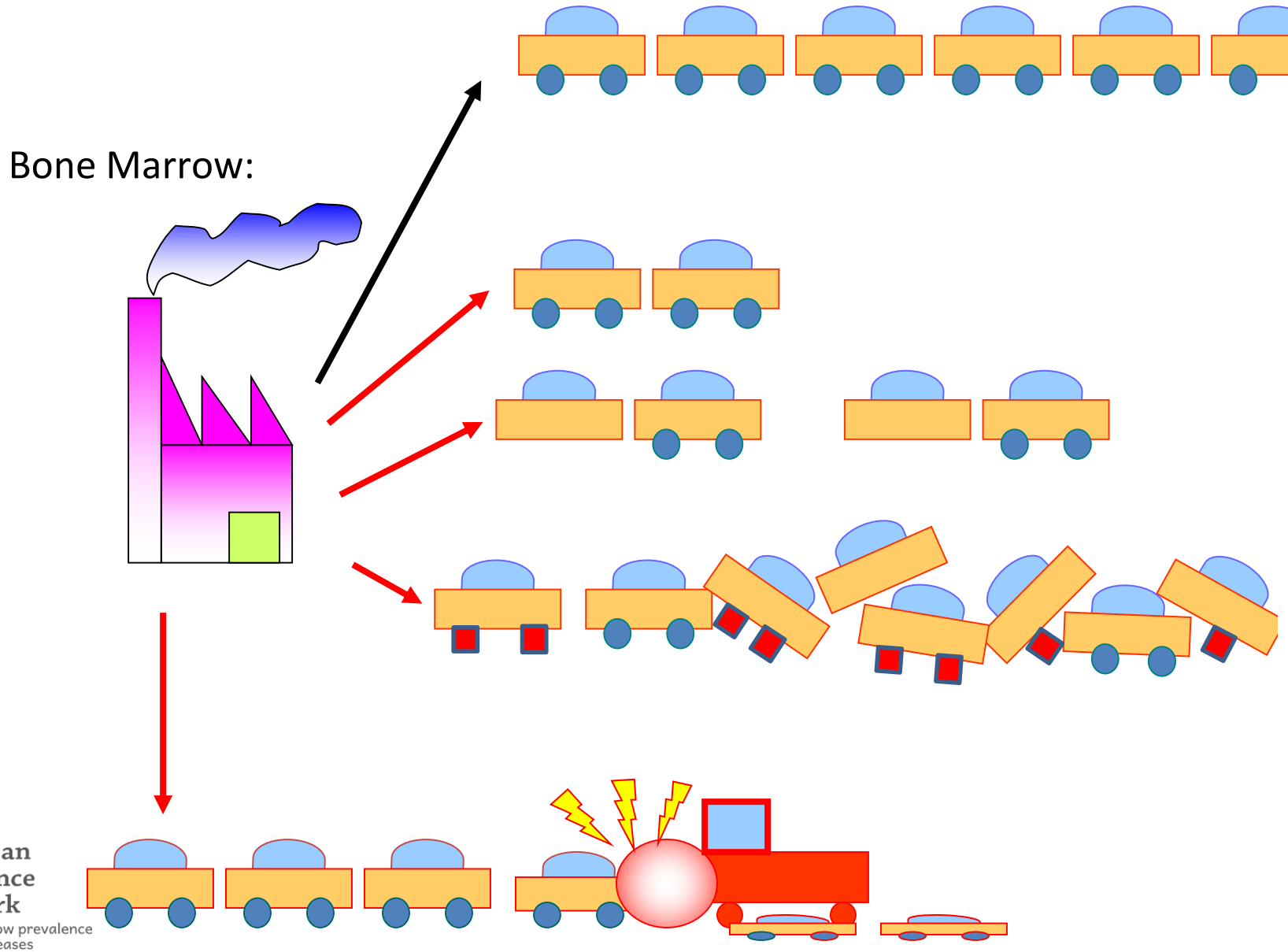


Reticulocytes



Mature red cell






Anemia

Jaundice

Hyperbilirubinemia

Splenomegaly

Iron overload

**Differential diagnosis!**

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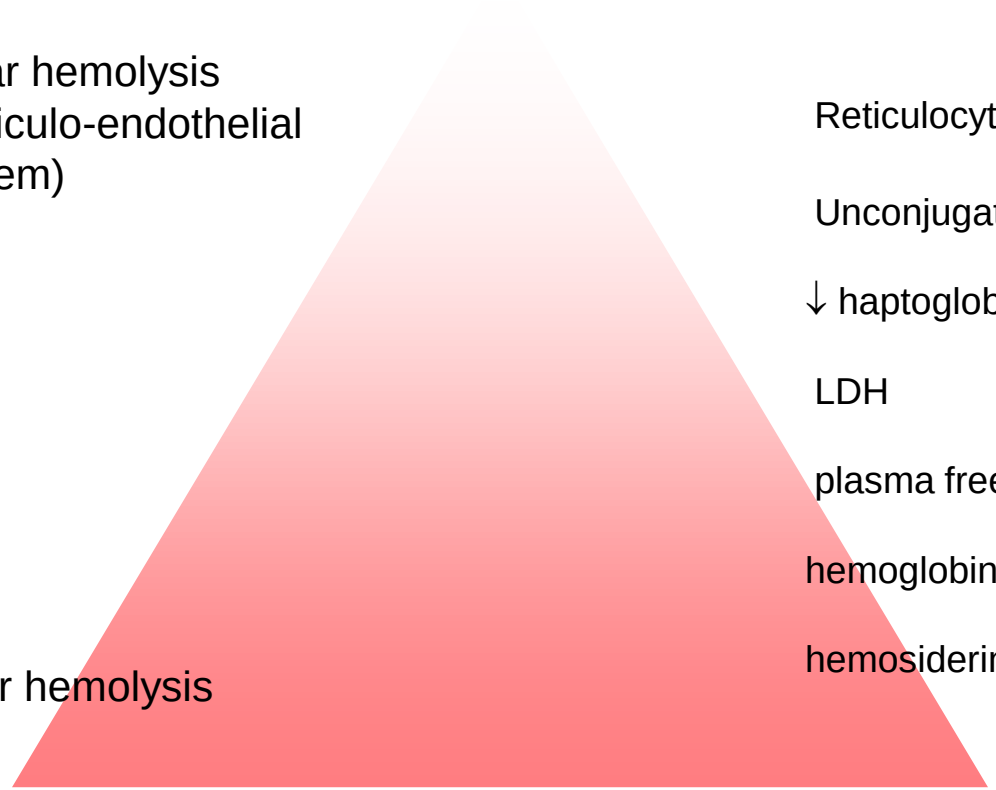
## Help from laboratory data

Extravascular hemolysis
(spleen, liver, reticulo-endothelial system)



Intravascular hemolysis

Reticulocytes

Unconjugated bilirubin

↓ haptoglobin

LDH

plasma free hemoglobin

hemoglobinuria

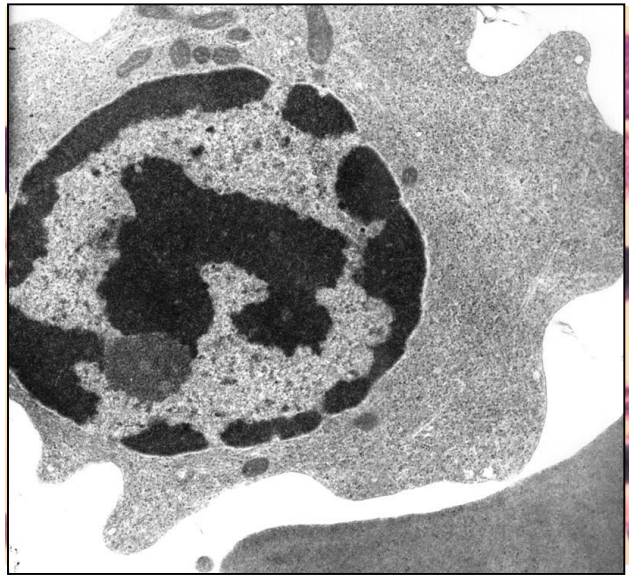
hemosiderinuria

## When to suspect a CDA?

- When in presence of hemolysis the increase of reticulocyte number is not proportional to the degree of anemia
- Presence of bone marrow abnormalities
- Iron overload due to both ineffective erythropoiesis and peripheral hemolysis

# Typical features in CDAI

| | |
|---|---|
| Transmission | Autosomal recessive transmission |
| Clinical picture | Mild – moderate anemia<br>Hydrops fopetalis, fetal demise if untreated |
| Physical examination | **10-20% cases non-hematological manifestations**<br>axial skeleton abnormalities (missing distal phalanges, syndactyly, lack of nail formations) |
| Laboratoy Investigations | **Blood count:** Hb ↓, MCV ↑<br>**RBC morphology:** anisocytosis and poikilocytosis<br>**BM examination:** binucleate macrocytic erythroblasts and internuclear bridging<br>**Electron microscopy**: 'Swiss cheese' or 'spongy' heterochromatin |
| Molecular investigations | Homozygote or compound het mutations in CDAN1 or C15orf41 genes<br>About 10% of cases no mutations in these genes<br>Sanger sequencing/ Clinical-grade NGS |



Roy N & Babbs C, 2019

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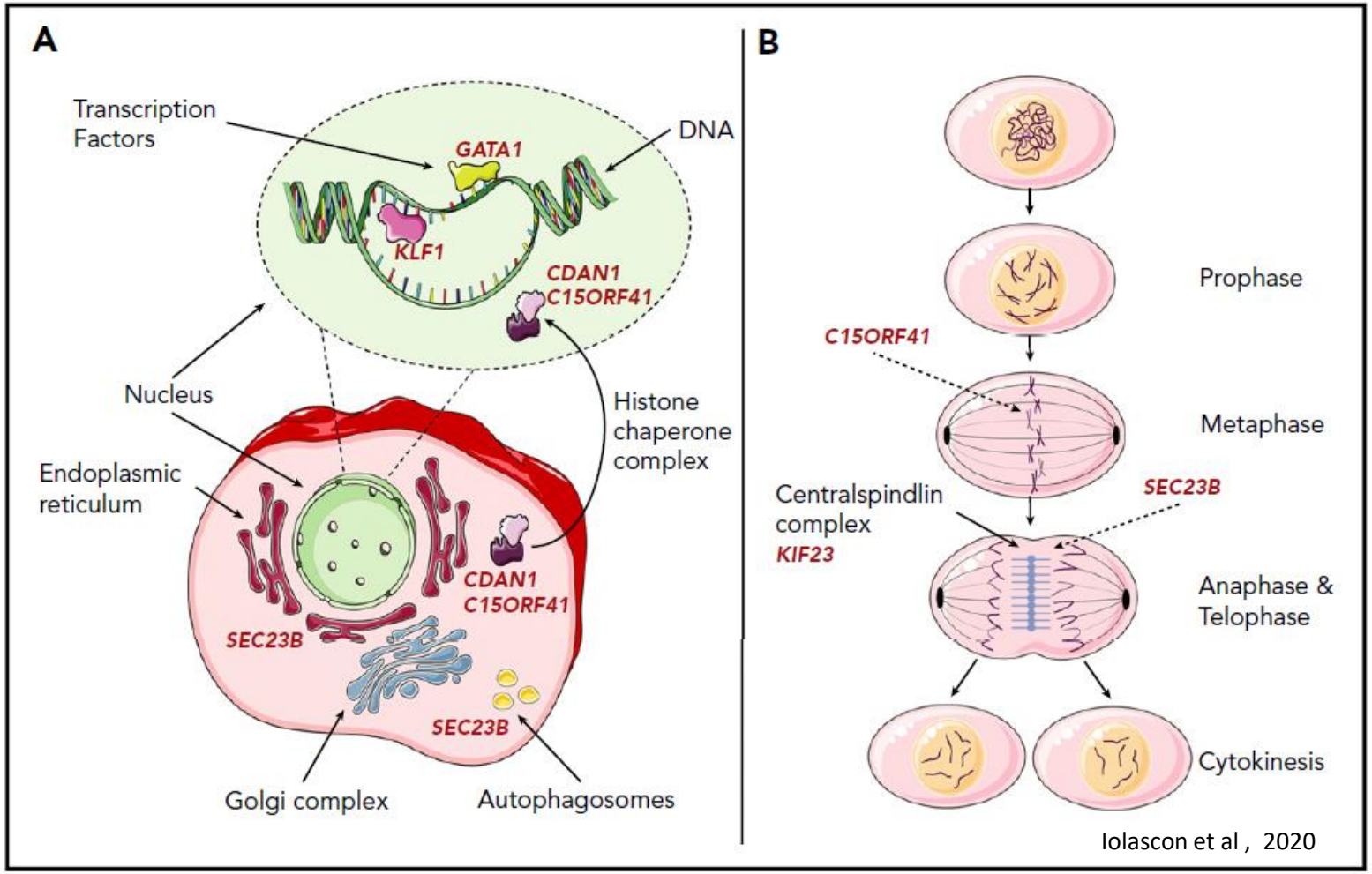
Impairment of mechanisms involved in DNA synthesis and chromatin assembly

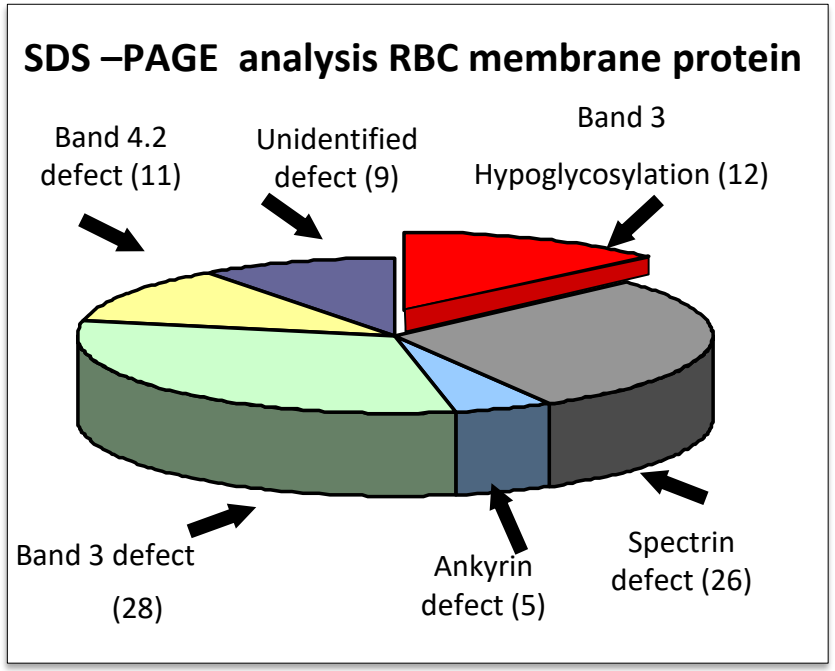



Iolascon et al , 2020

Dgany et al, 2002; Babbs et al, 2013; Roy & Babbs, 2019

# Typical features in CDAII

| | |
|---|---|
| Clinical picture | Anemia (mild to severe)<br>Hydrops fopetalis, fetal demise if untreated |
| Laboratoy Investigations | **Blood count:** ↓ Hb<br>**RBC morphology:** anysocytosis and poikilocytosis,<br>**BM examination:** Bi-nuclearity or multinuclearity in 10-40% of erythroblasts<br>Electron microscopy: Double membranes close to the outer cell membrane due to a defective ER clerance during erythroid maturation |
| Biochemica l investigations | Relevant hypoglycosylation of erythrocyte membrane proteins band 3 and 4.5 that carry altered N-glycans with truncated poly-lactosamine structures |
| Molecular investigations | Homozygote or compound het mutations in SEC23B<br>In a small proportion of cases only 1 het or no mutations detected in SEC23B<br>Sanger sequencing/ Clinical-grade NGS |

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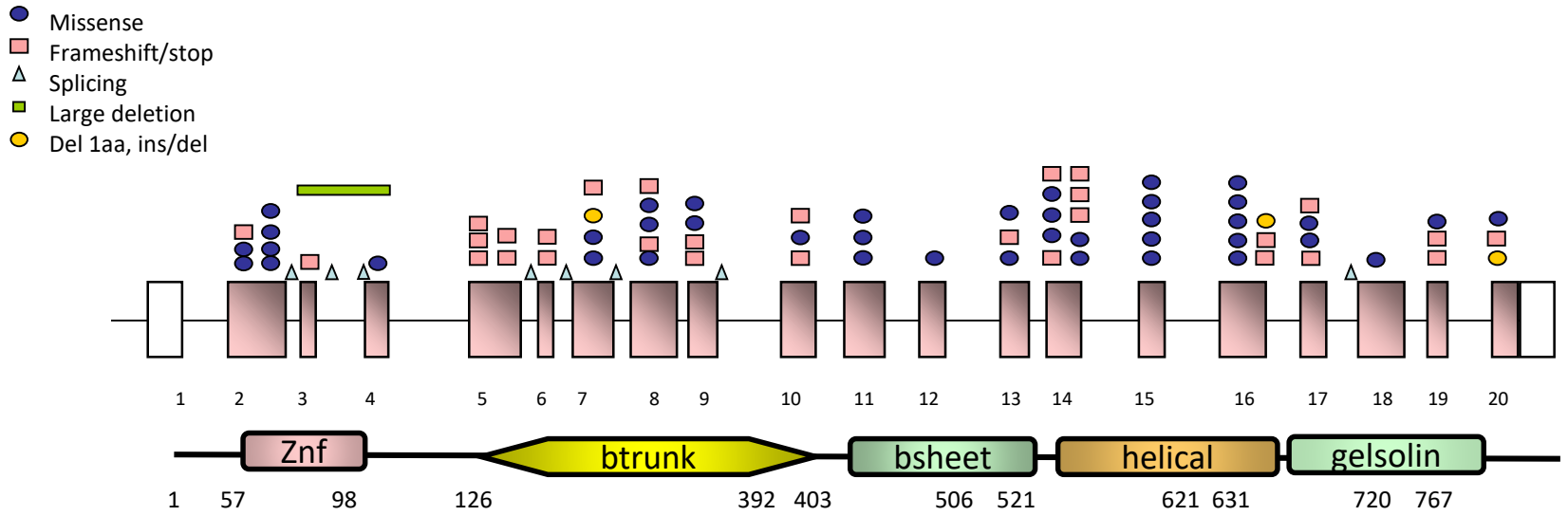
# Molecular basis of CDAII




**ORF: 2304bp**

***SEC23B***

Bianchi et al 2009, Schwartz, et al 2009

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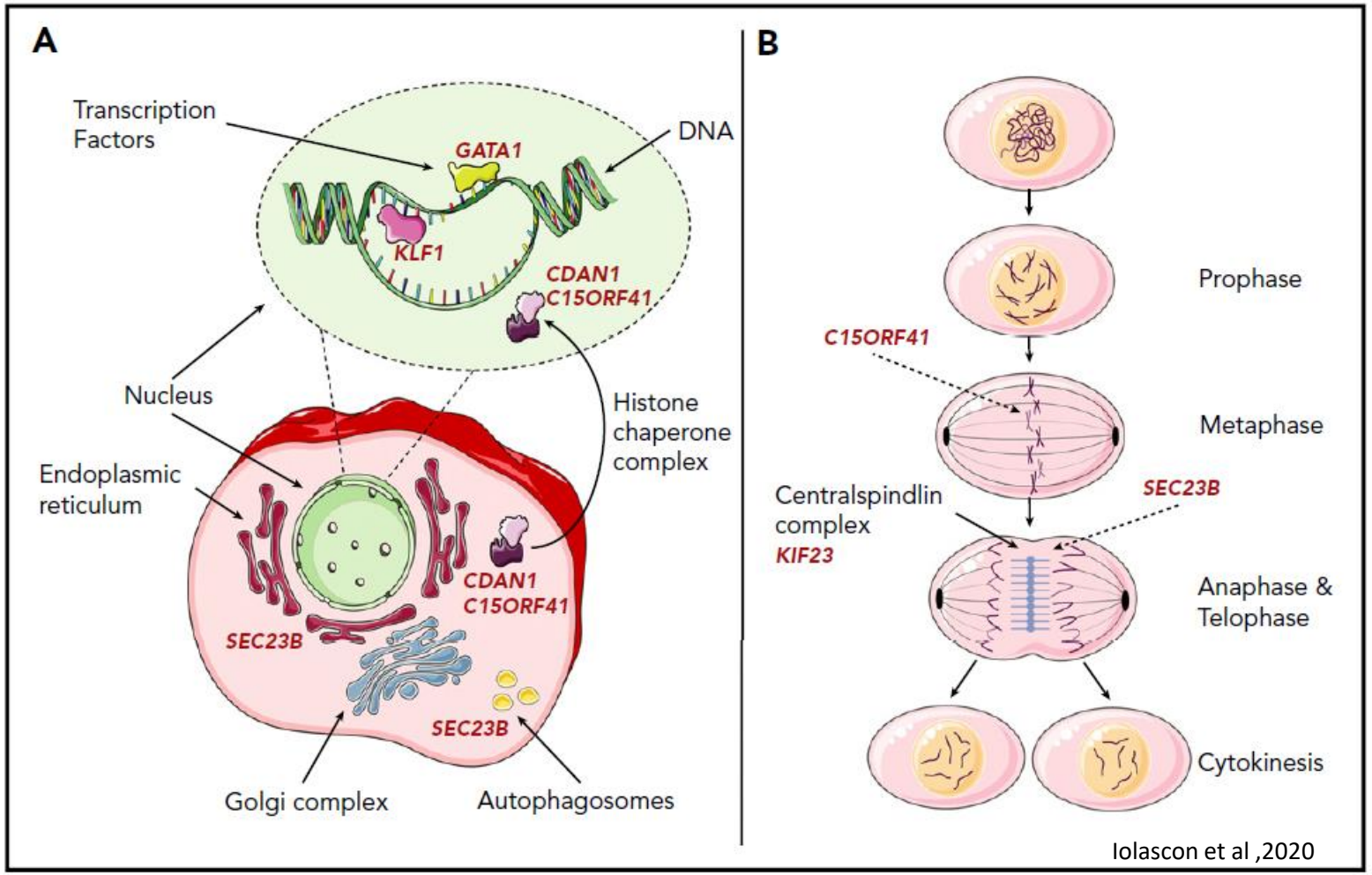
# Molecular basis of CDAII




Iolascon et al ,2020

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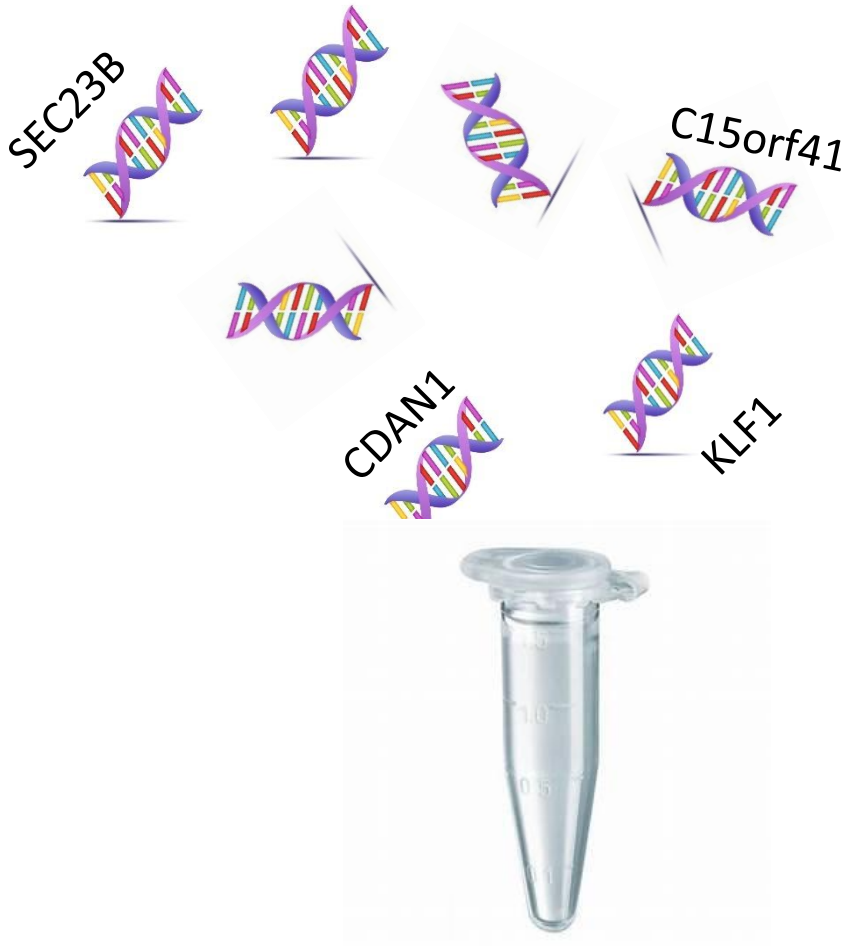
# New diagnostic approaches: Next generation targeting sequencing



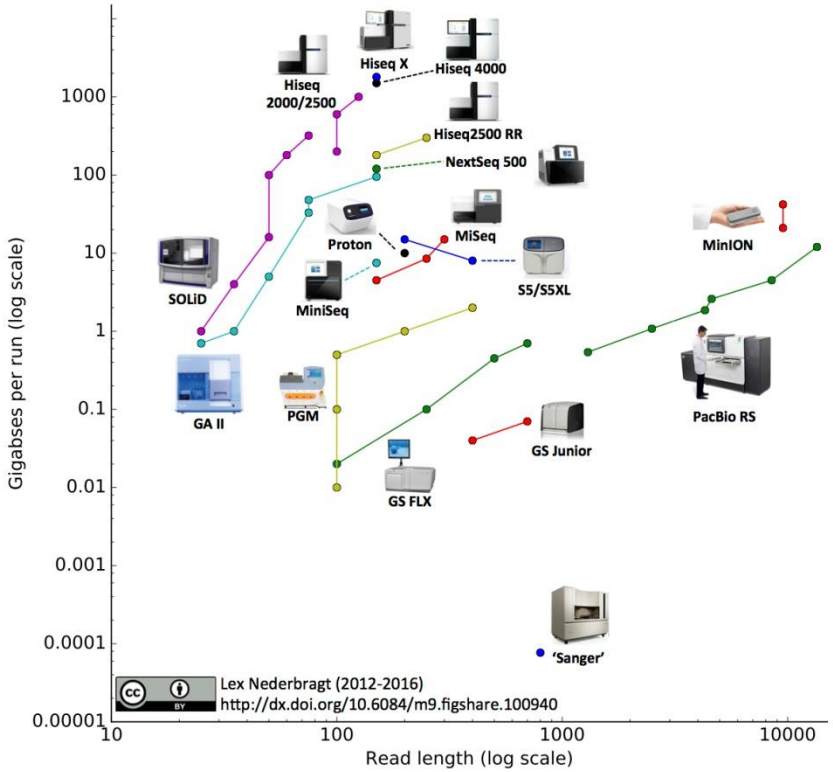




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Table 1. Recent studies performed by next-generation sequencing technologies in patients with hemolytic anemias.

| Reference | Method | N. of genes analyzed | N. of cases studied with CHA | PKD diagnosis | New diagnosis and number and type of mismatched diagnoses |
|---|---|---|---|---|---|
| 15 | t-NGS | 35 | 36 | 2 | 2 new PKD |
| 28 | t-NGS | 55 | 43 | 8 | 8 new PKD |
| 29 | WES | n.a. | 4 | 4 | 4 new PKD |
| 30 | t-NGS | 76 | 21[a] | 6 | 3 new PKD<br>2 CDA→ PKD<br>1 DBA→PKD |
| 27 | t-NGS | 76 | 21[b] | 6 | 4 new PKD<br>2 CDA→PKD |
| 25 | t-NGS | 34 and 71 | 74[c] | 7 | 7 CDA→PKD |
| 23 | t-NGS | 33 | 57 | 3 | 2 new PKD<br>1 CDA→PKD |

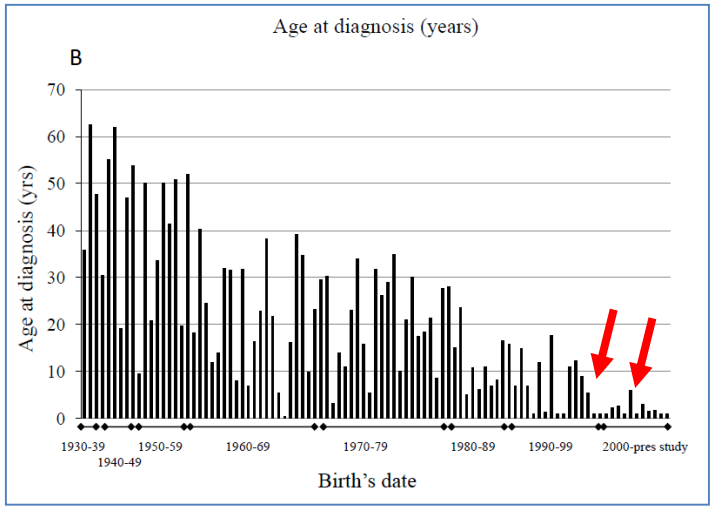
Bianchi &Fermo, 2020



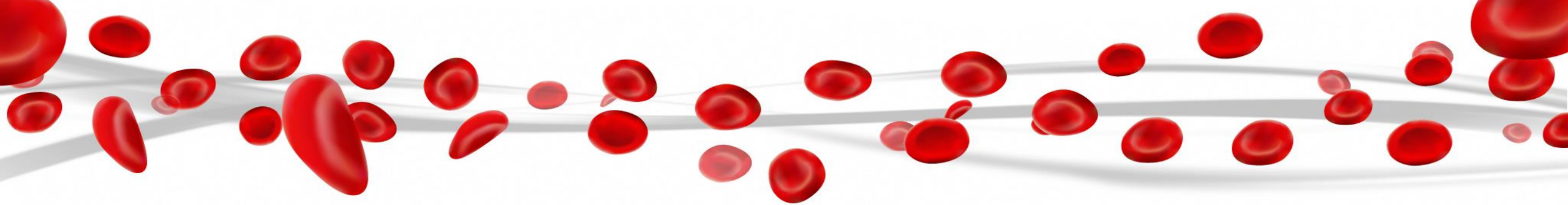
Roy et al, 2016; Russo et al 2018, Shefer Averbuch et al 2018; Jamwal et al 2018; Qin et al 2020; Kedar et al 2019;

# Add value of molecular investigations: Take home messages

Drop down of age at diagnosis




- ❑ Reduction of misdiagnosis
- ❑ Genetic counselling
- ❑ Increase the awarness in these disorders
- ❑ Understanding the phatophysiology
- ❑ New disease-specific therapies



# Thank you!

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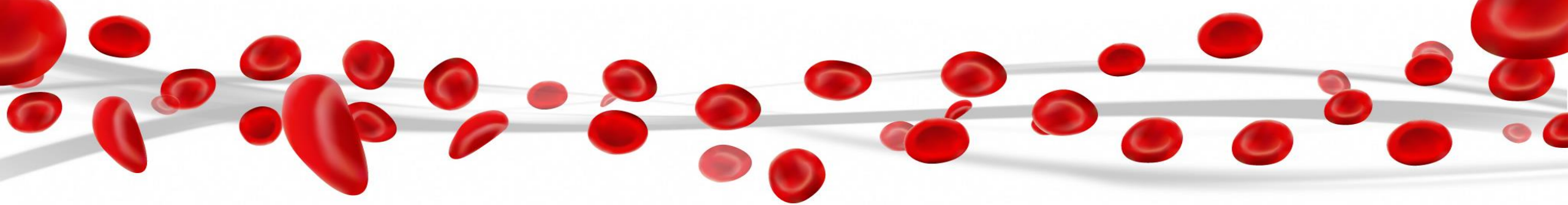

**Please, write your questions in the chat**

**we are going to answer at the end of the presentations.**

**Next presentation: Roberta Russo, *Clinical Aspects***

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# Congenital dyserythropoietic anemias: clinical aspects

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CEINGE - Advanced Biotechnologies, Naples
ERN-EuroBloodNet subnetwork: RBC
Red blood cell defects, Bone marrow failure
May 25, 2021

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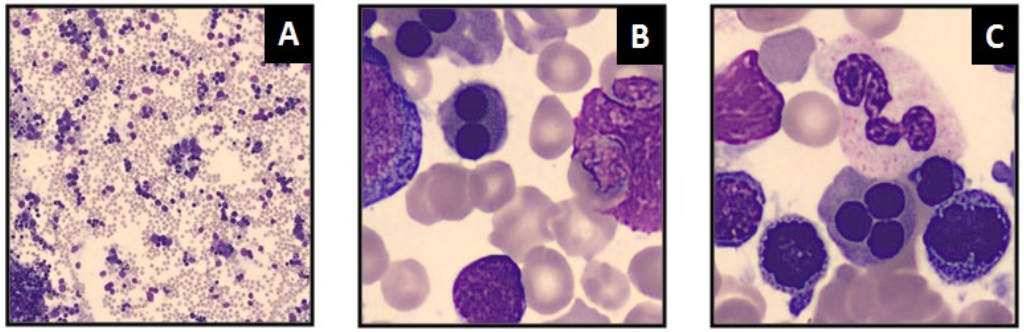
# Conflicts of interest

**Nothing to disclose**

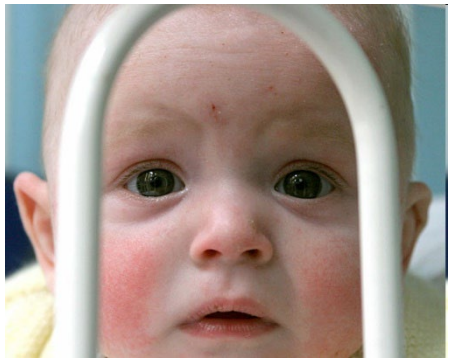
# Congenital Dyserythropoietic Anemias

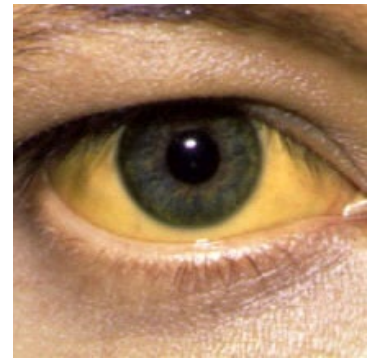
✓ **CDAs** are **Mendelian** (i.e. single-gene) diseases affecting the normal differentiation-proliferation pathway of the erythroid lineage

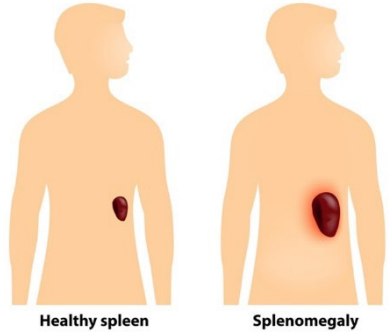



**Erythroid hyperplasia with specific morphological alterations of late erythroblasts**

- **Hemolytic anemia**
- **Reduced retics count**
- **Jaundice**
- **Splenomegaly**
- **Hemosiderosis**
- **Gallstones**
- **Transfusion dependence (≈ 20%)**





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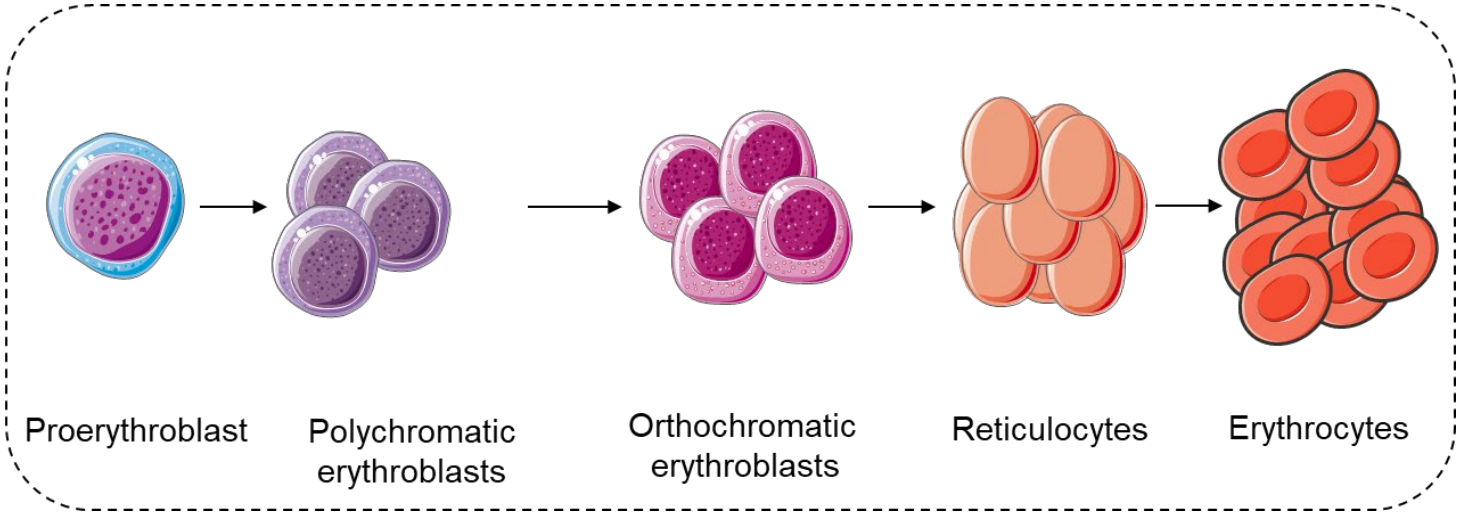
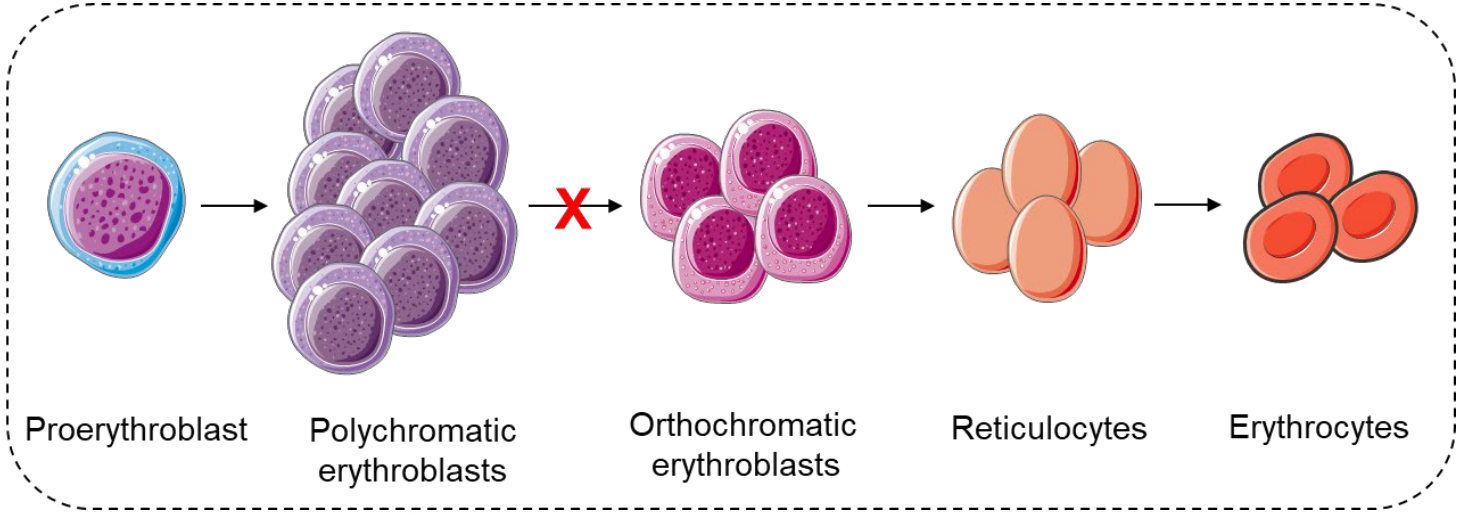
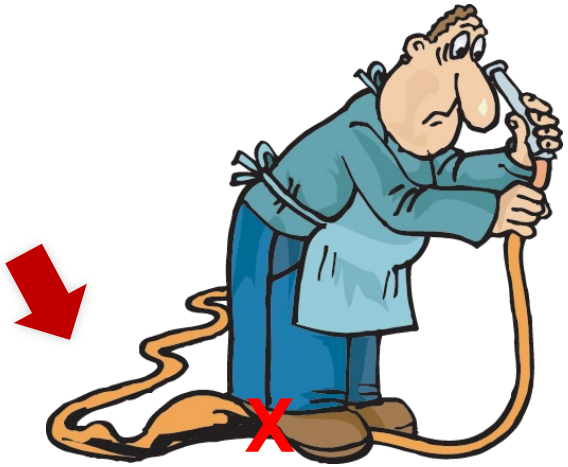
# Erythroid hyperplasia



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*Iolascon A, Andolfo A, Russo R. Blood 2020; Russo R, et al. 2021*

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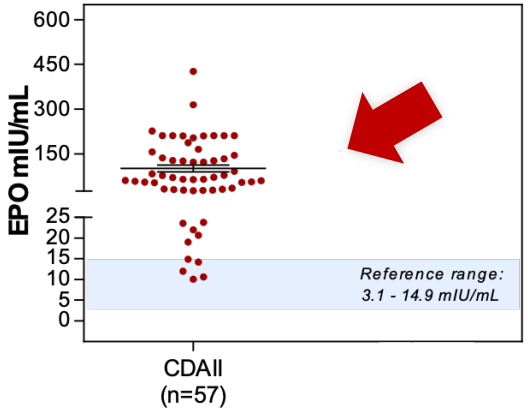
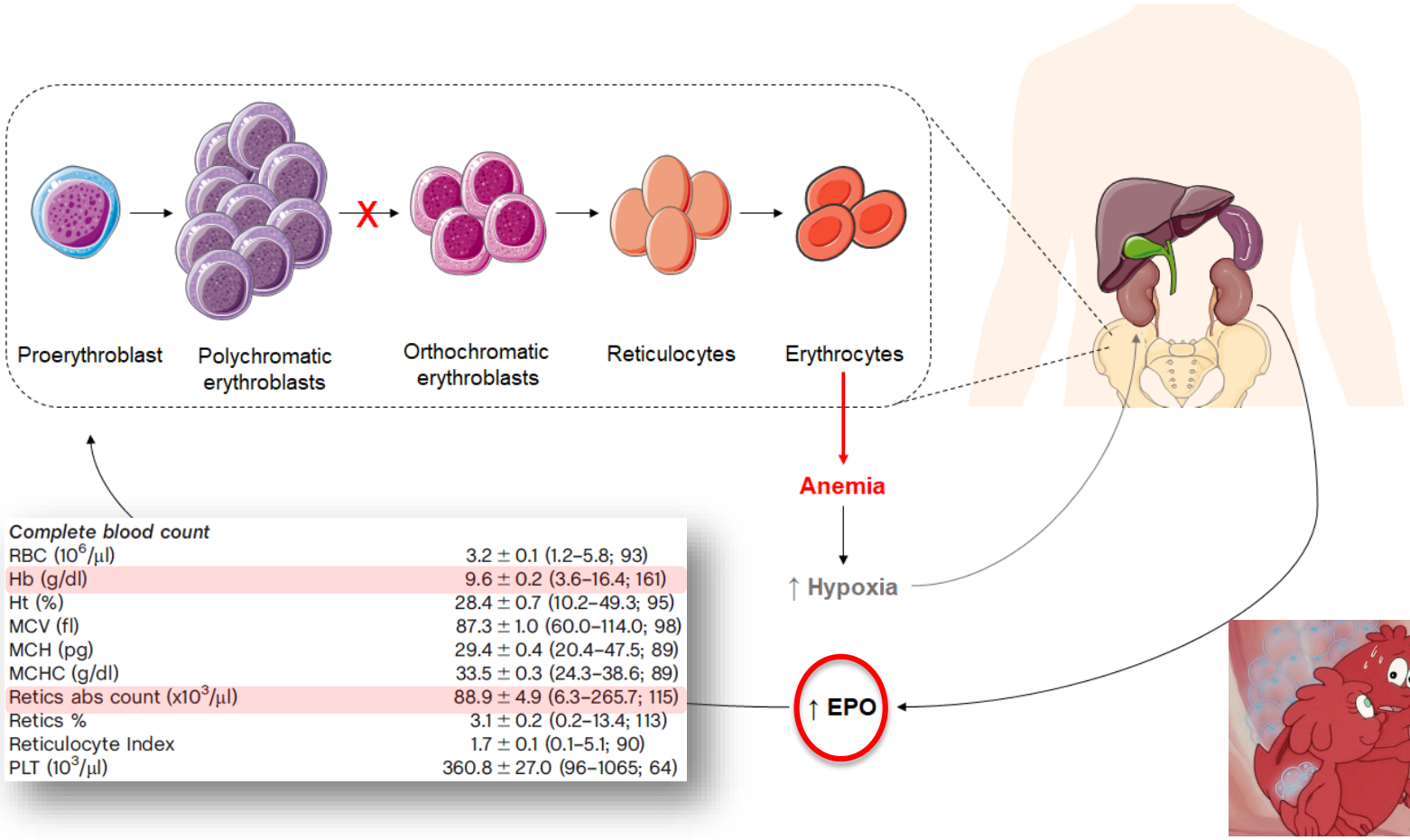
# Physiopathology of CDAs (at systemic level)

- Hemolytic anemia
- **Reduced retics count**
- Jaundice
- Splenomegaly
- Hemosiderosis
- Gallstones
- TD (≈ 20%)

✓ Anemia with reduced reticulocyte count

✓ **EPO** is not able to increase the production of RBCs




| *Complete blood count* | |
|---|---|
| RBC ($10^6$/µl) | 3.2 ± 0.1 (1.2–5.8; 93) |
| Hb (g/dl) | 9.6 ± 0.2 (3.6–16.4; 161) |
| Ht (%) | 28.4 ± 0.7 (10.2–49.3; 95) |
| MCV (fl) | 87.3 ± 1.0 (60.0–114.0; 98) |
| MCH (pg) | 29.4 ± 0.4 (20.4–47.5; 89) |
| MCHC (g/dl) | 33.5 ± 0.3 (24.3–38.6; 89) |
| Retics abs count (x$10^3$/µl) | 88.9 ± 4.9 (6.3–265.7; 115) |
| Retics % | 3.1 ± 0.2 (0.2–13.4; 113) |
| Reticulocyte Index | 1.7 ± 0.1 (0.1–5.1; 90) |
| PLT ($10^3$/µl) | 360.8 ± 27.0 (96–1065; 64) |



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# Physiopathology of CDAs (at systemic level)

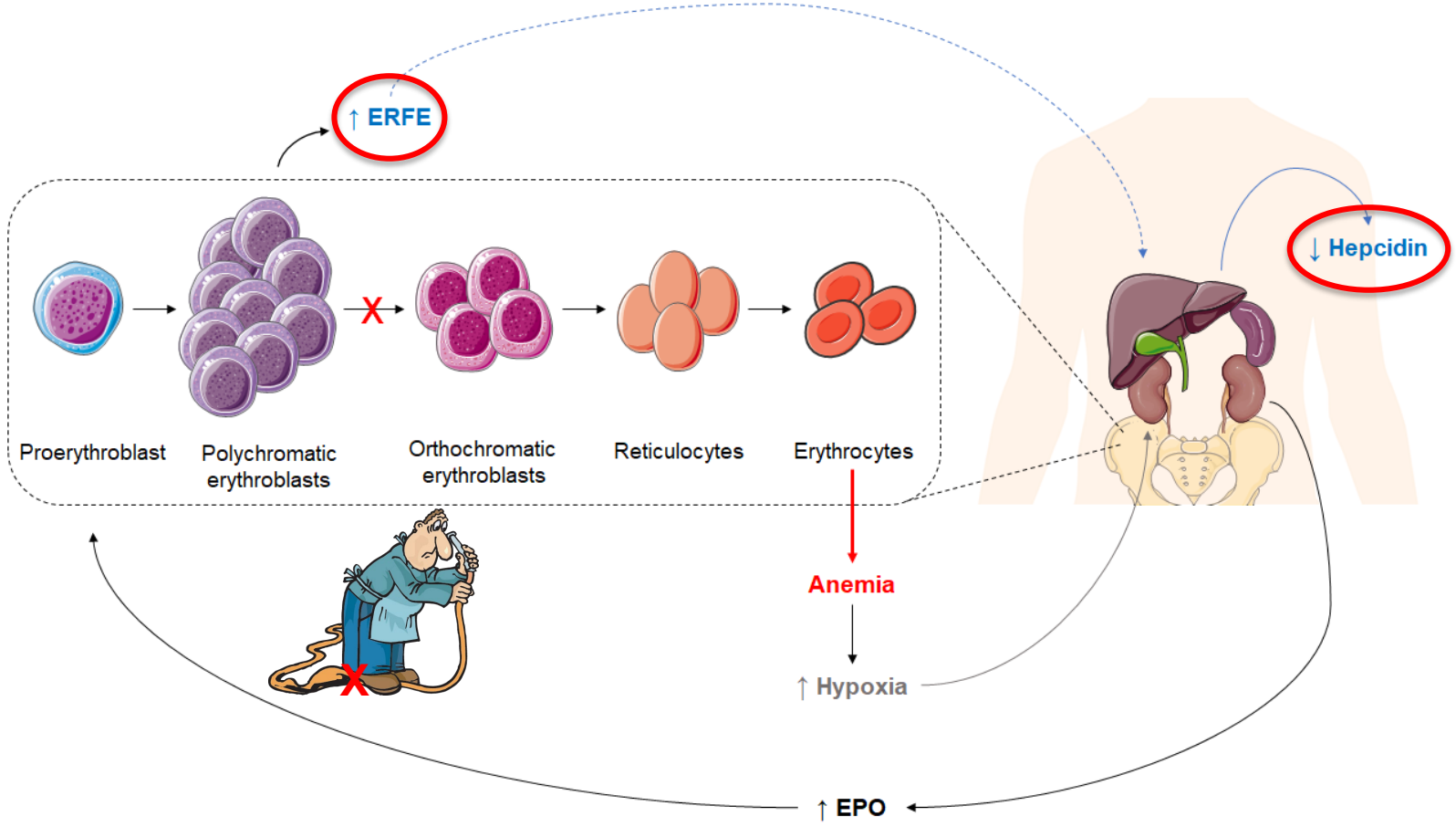
- Hemolytic anemia
- Reduced retics count
- Jaundice
- Splenomegaly

- **Hemosiderosis**
- Gallstones
- TD (≈ 20%)

✓ Increased levels of erythroferrone (**ERFE**)

✓ Reduced expression of hepatic hormone **hepcidin**

✓ It regulates the iron absorption and tissue distribution

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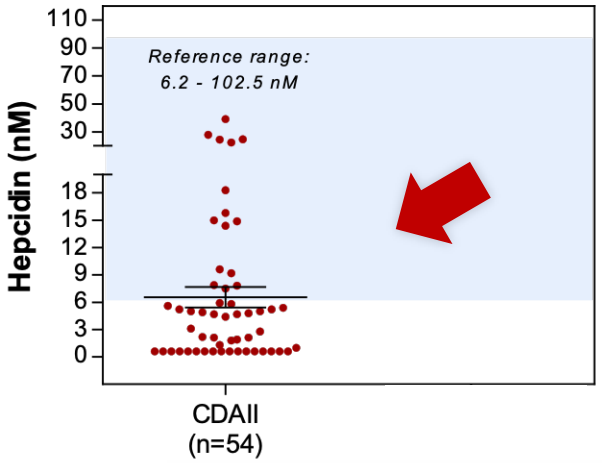
# Iron overload

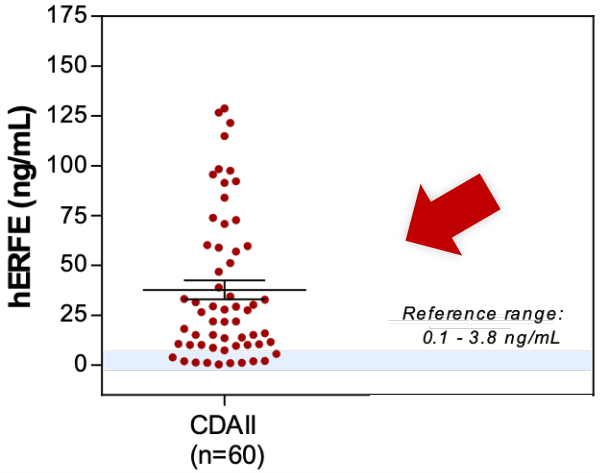
- Hemolytic anemia
- Reduced retics count
- Jaundice
- Splenomegaly

- **Hemosiderosis**
- Gallstones
- TD (≈ 20%)

## Reduced hepcidin levels




## Increased ERFE levels




| *Biochemical, laboratory data and iron balance* | |
|---|---|
| Hypoglicosylation of Band 3 (Yes/No) | 78 (95.1)/4 (4.9) |
| Total bilirubin (mg/dl) | 2.8 ± 0.2 (0.2–12.7; 108) |
| Ferritin (ng/ml) | 464.8 ± 55.9 (15–3500; 133) |
| Transferrin saturation (%) | 62.4 ± 4.2 (12.0–113.0; 43) |
| Iron (µg/dl) | 156.0 ± 9.1 (30.0–374.0; 64) |
| sTfR (mg/l) | 5.4 ± 1.2 (1.4–45.3; 35) |

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*Russo R, et al. Am J Hematol. 2014; Russo R, et al. 2021*

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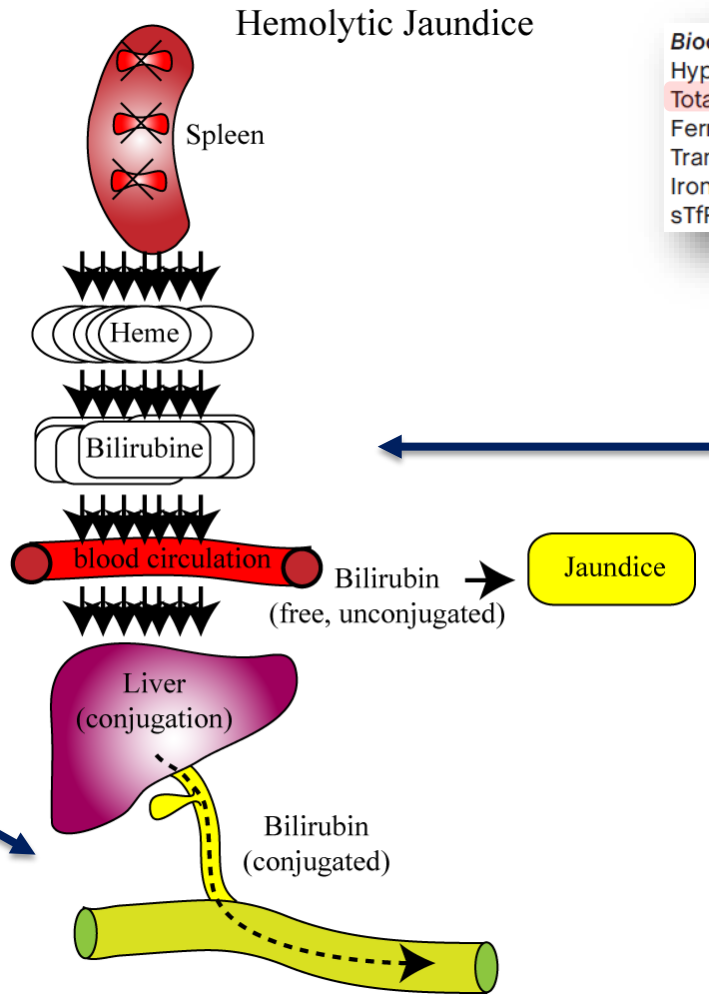
# Physiopathology of CDAs (at systemic level)

- **Hemolytic anemia**
- Reduced retics count
- **Jaundice**
- **Splenomegaly**

- Hemosiderosis
- **Gallstones**
- TD (≈ 20%)

Abnormal red blood cells are destroyed into the **spleen**

**Splenomegaly**

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| sTfR (mg/l) | 5.4 ± 1.2 (1.4–45.3; 35) |

Destroyed red blood cells increase the bilirubin levels

**Jaundice**

Bilirubin accumulates in the **gallbladder**

**Gallstones**

*Russo R, et al. Am J Hematol. 2014*

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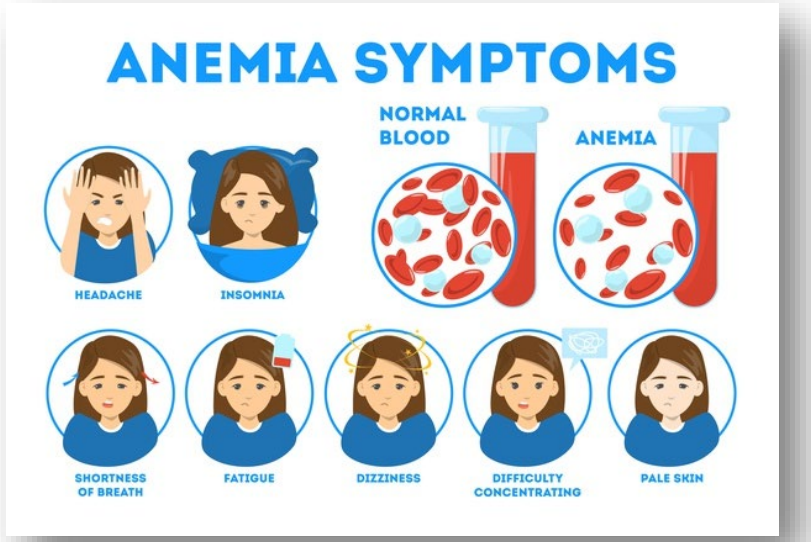
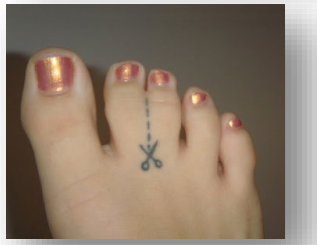
## Clinical features:

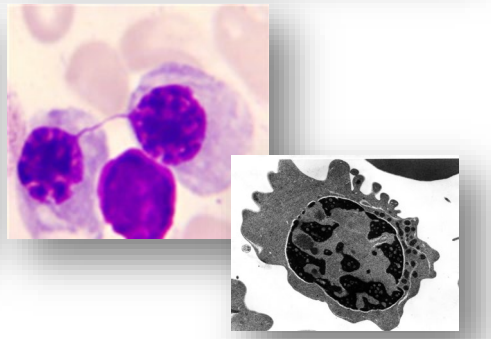
- severe or moderate anemia (generally ***macrocytic***) with neonatal appearance
- Jaundice
- Splenomegaly
- Hemosiderosis




## Morphologic body abnormalities:

- 10% of patients
- skeletal malformations
- syndactyly in hands or feet
- absence of nails or supernumerary toes





European Reference Network for rare or low prevalence complex diseases

Network Hematological Diseases (ERN EuroBloodNet)

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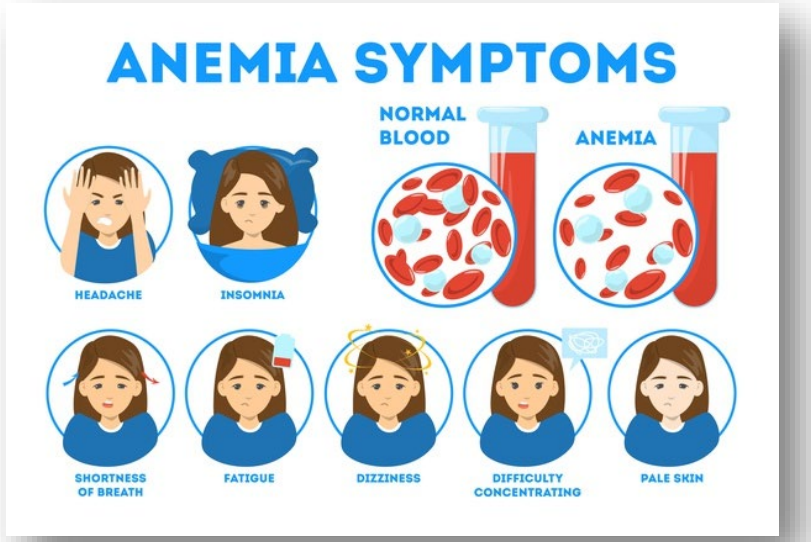
*Roy NB, Babbs C. BJH 2019*
*Iolascon A, Andolfo A, Russo R. Blood 2020*

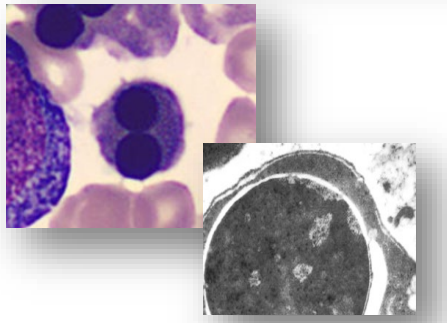
Patients Webinars

EuroBloodNet

## Clinical features:

- ✓ Average age of onset symptoms: 3.7 ± 0.6 y
- ✓ Mean age at diagnosis: 22.2 ± 1.7 y
- ✓ Normocytic mild anemia:
  - **Hb 9.6** ± 0.2 g/dL
  - **MCV 87.3** ± 1.0
- ✓ Reticulocyte index: 1.7 ± 0.1
- ✓ Mean serum ferritin: 464.8 ± 55.9 ng/mL
- ✓ Splenomegaly: 102/122, **83.6% of patients**
- ✓ Transfusion dependency: 25/126, **19.8% of patients**



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*Russo R. et al. Am J Hematol. 2014*

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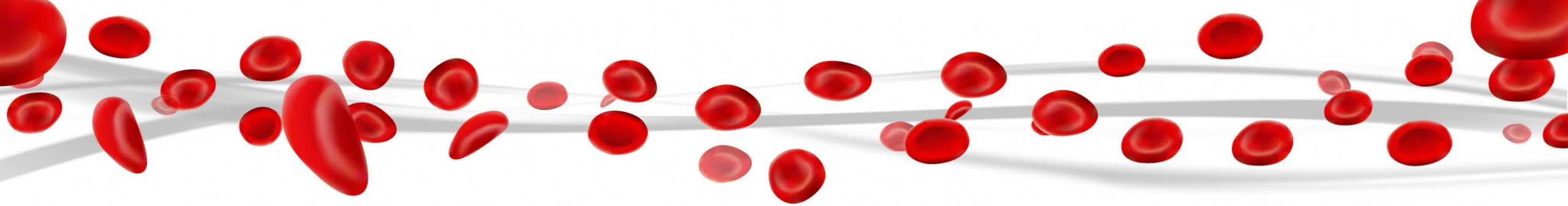

Please, write your questions in the chat

we are going to answer at the end of the presentations.

Next presentation: Noémi Roy, *Current and future therapeutic perspectives*

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