Cosa c’è di nuovo per trattare l’anemia
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Patogenesi delle Anemie
Sideropeniche

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Disclosure

- Member of advisory board for:
  - Novartis
  - Sanofi Genzyme
  - Celgene
Agenda

- Global burden of anemia
- Definition Iron Deficiency (ID) and Iron Deficiency Anemia (IDA)
- Iron Metabolism
- Causes of ID/IDA
The global burden of anaemia

- 32.9% global anaemia prevalence
- 68.4 mio years lived with disability (YLD, anaemia)
  - i.e. 8.8% of total for all conditions

Prevalence of anaemia by aetiology

ID and ID(A): Definition

Iron Deficiency
• Depleted iron stores

Iron Deficiency Anaemia
• Depleted iron stores
• Haemoglobin (Hb) concentration falls below defined lower limit (12g/dl for women, 13 g/dl for men)

Absolute Iron Deficiency (no iron stores)
• Low Transferrin Saturation (TSAT<20%)
• Serum ferritin low (<20ng/ml – or <100ng/ml in case of inflammation)
• Elevated Serum Transferrin Receptor (sTfR)

Functional Iron Deficiency (full iron stores but ID in erythroid bone marrow)
• Low TSAT (TSAT<20%)
• Serum ferritin normal or elevated (>20 ng/ml – or >100ng/ml in case of inflammation)
• Normal sTfR

Adapted from Hush R. and Schaefer R. Pocket Atlas Special. Thieme 2006 and Prof. IY. Beguin oral communication. Parallel Symposia at EHA congress 2014, Milan, Italy
ID and ID(A): Relationship with anaemia

Schematic representation only. Areas do not reflect the real magnitude of the problem.

ID and ID(A): Causes

Iron Deficiency

Absolute Iron Deficiency

Functional Iron Deficiency

Blood Loss
- Heavy menstrual bleeding
- Delivery
- Gastrointestinal disorders/bleeding
- Surgery
- Blood donation
- Haemodialysis

Decreased Iron Intake
- Poor diet
- Vegetarian/vegan diet
- Disease-related anorexia
- Eating disorders

Increased Iron Demand
- Infancy
- Adolescence
- Pregnancy
- Endurance sport

Decreased Iron Absorption and utilisation/released
- Inflammatory bowel disease
- Chronic or malignant disease
- Interaction with food components
- Concomitant intake of other drugs
- Malabsorption

References:
Agenda

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- Causes of ID/IDA
Physiological iron turn-over

Dietary iron

Utilisation

Plasma transferrin (3mg)

Muscle (myoglobin) (300mg)

Liver parenchyma (1000mg)

Storage iron

Duodenum (average, 1-2 mg/day)

Utilisation

Other iron-containing enzymes (100mg)

Bone marrow (300mg)

Sloughed mucosal cells, desquamation, menstruation, other blood loss

25 mg/day

Circulating erythrocytes (Hb) (1800mg)

Reticuloendothelial macrophages (600mg)

Iron loss 1–2 mg/day

The iron cycle

Iron absorption

Enterocyte
- 1–2 mg/day
- Hepcidin-regulated
- Balanced by iron losses (1–2 mg/day)
- Reduced in inflammation
- Increased in iron deficiency

Iron recycling

Macrophage
- 20–30 mg/day
- Hepcidin-regulated
- Balanced by erythroid request
- Reduced in inflammation
- Increased in iron deficiency

Hepcidin: The master regulator of iron homeostasis

Liver 1000 mg

Hepatocytes

Duodenum

Plasma Fe-Tf

20–25 mg/d

1–2 mg/d

1–2 mg/d

Not controlled

Controlled

Erythrocytes

Erythroid marrow

Splenic and other macrophages

≈600 mg

≈2000 mg

Ganz T, Physiol Rev 2013
Hepcidin is the master regulator of systemic iron homeostasis

- The liver peptide hepcidin regulates intestinal iron absorption and iron release from storage cells by binding ferroportin, causing its internalization and degradation, and thus exerting a general inhibitory effect on iron release in the body.

FPN = ferroportin.

Macrophage

**Hepcidin upregulation: two pathways**

**Iron-dependent pathway**

**Inflammatory pathway**

LPS = lipopolysaccharide.

* Indicates proteins whose inactivation causes iron overload.
Hepcidin downregulation: multiple pathways

Hypoxia, iron deficiency
- Hypoxia, iron deficiency
- BMP6
- s-HJV
- TWSG1
- GDF15

Erythropoietic expansion
- Erythropoietic expansion
- Erythroferrone
- BMP6
- TMPRSS6
- HJV
- HFE
- HJVR
- HIF-1α

(Epo)
Hepcidin regulation by anaemia

Ganz T, Physiol Rev 2013
Erythroferrone (ERFE) the newly identified erythroid regulator

Proposed mechanism of action

Kautz L, Nat Genet 2014

Identification of erythroferrone as an erythroid regulator of iron metabolism

Kautz L, Nat Genet 2014
‘Traditional’ view of iron – a critical element in oxygen delivery (erythropoiesis)

- Pluripotent stem cell
- Burst-forming unit-erythroid cells
- Colony-forming unit-erythroid cells
- Proerythroblasts
- Erythroblasts
- Reticulocytes
- Red blood cells

- Erythropoietin dependence
- Iron dependence

About 3–4 days
About 10–13 days
About 21 days
About 1–2 days

Adapted from Besarab A, et al. The Oncologist 2009;14:22–33
Globins, iron, and heme play a critical role in MCV determination.

**Mean MCV**
- 0 day 131 fL
- 7 day 99 fL
- 14 day 86 fL

Data obtained from erythroid cultures (Drs. Iolascon and De Falco). Graphics courtesy of Prof. Achille Iolascon.
**Characteristics of Microcytosis**

✓ **RBC**: Microcytosis hypochromia
   - reduced size and reduced Hb content of red blood cells, as inferred by erythrocyte indexes

### Normal values for age

<table>
<thead>
<tr>
<th>Age</th>
<th>MCV (fl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>At born</td>
<td>110-128</td>
</tr>
<tr>
<td>5-24 months</td>
<td>80-85</td>
</tr>
<tr>
<td>2-6 years</td>
<td>75-90</td>
</tr>
<tr>
<td>6-12 years</td>
<td>78-95</td>
</tr>
<tr>
<td>&gt;12 years</td>
<td>80-100</td>
</tr>
</tbody>
</table>

**MCH**: <26 pg (n.v. 27-30)

**MCHC**: <30 g/dl (n.v. 31-37)
Differential diagnosis of the most common forms of microcytosis

<table>
<thead>
<tr>
<th></th>
<th>Nutritional deficiency</th>
<th>Deficit of absorption</th>
<th>Thalassemia heterozygotes</th>
<th>ACD</th>
<th>ACD+iron deficiency</th>
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</thead>
<tbody>
<tr>
<td>Hb</td>
<td>-</td>
<td>-</td>
<td>= / -</td>
<td>-</td>
<td>--</td>
</tr>
<tr>
<td>MCV</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>GR</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>--</td>
</tr>
<tr>
<td>RDW</td>
<td>=</td>
<td>=</td>
<td>= / +</td>
<td>= / +</td>
<td>+</td>
</tr>
<tr>
<td>Reticulocytes</td>
<td>-</td>
<td>-</td>
<td>= / +</td>
<td>= / +</td>
<td>= / + / -</td>
</tr>
<tr>
<td>IS</td>
<td>- / --</td>
<td>- / --</td>
<td>=</td>
<td>= / -</td>
<td>-</td>
</tr>
<tr>
<td>Ferritin</td>
<td>= / -</td>
<td>= / +</td>
<td>=</td>
<td>=</td>
<td>= / -</td>
</tr>
<tr>
<td>FEP</td>
<td>= / +</td>
<td>= / +</td>
<td>=</td>
<td>=</td>
<td>= / +</td>
</tr>
<tr>
<td>sTfR</td>
<td>+</td>
<td>+</td>
<td>=</td>
<td>=</td>
<td>= / +</td>
</tr>
<tr>
<td>CHr</td>
<td>-</td>
<td>-</td>
<td>= / -</td>
<td>-</td>
<td>--</td>
</tr>
<tr>
<td>Oral response</td>
<td>YES</td>
<td>NO</td>
<td>NO</td>
<td>Not to be expected</td>
<td>Partial</td>
</tr>
<tr>
<td>Iv response</td>
<td>YES</td>
<td>YES</td>
<td>NO</td>
<td>Not to be expected</td>
<td>Partial</td>
</tr>
<tr>
<td>Inheritance</td>
<td>Acquired</td>
<td>Acquired / multifactorial</td>
<td>AR</td>
<td>Multifactorial</td>
<td>Multifactorial</td>
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<tr>
<td>Suggested therapy</td>
<td>Oral iron</td>
<td>Etiological therapy / iv injection if severe anemia</td>
<td>Not required</td>
<td>Etiological therap yif possible (EPO, iv iron)</td>
<td>Etiological therap + oral iron</td>
</tr>
</tbody>
</table>

Iolascon A et al., 2013
Causes of Iron Deficiency Anaemia

- Blood loss
- Limited supply (poor diet)
- Increased requirements
- Iron malabsorption

IRIDA

Hereditary

Acquired
Unexplained or Refractory Acquired Iron-Deficiency Anaemia (IRIDA)

- *Helicobacter pylori*
- Celiac disease
- Autoimmune atrophic gastritis
Helicobacter pylori Infection

- In recent years, *H. pylori* has been implicated in several studies as a cause of IDA refractory to oral iron treatment
  
  - Favorable response to *H. pylori* eradication

- Mechanisms: Occult GI bleeding? Alterations in intragastric pH and ascorbic acid concentration? Induction of IL-1β and TNF-α, (inhibitors of parietal cell function)? Induction of parietal cell apoptosis?

- Diagnosis: IgG antibody screening, urease breath test

Celiac Disease

- Celiac disease is a common nonbleeding gastrointestinal condition that may result in refractory IDA\(^1\)
  - Celiac disease accounts for 5%–6% of unexplained IDA cases
  - Approximately 50% of patients with subclinical celiac disease develop IDA

- Diagnosis: Anti-tissue transglutaminase antibodies and/or anti-endomysial antibodies

Autoimmune Atrophic Gastritis

- Autoimmune atrophic gastritis, or atrophic body gastritis, is associated with chronic idiopathic IDA with no evidence of gastrointestinal blood loss
- Iron deficiency may develop many years before the depletion of vitamin $B_{12}$ stores
- Possible role of $H. pylori$ in the pathogenesis of autoimmune gastritis due to antigenic mimicry of $H^+K^+$-ATPase
- Diagnosis: Serum gastrin, parietal cell antibodies

Acquired IRIDA

- Blood loss, insufficient dietary iron intake, and increased iron requirements are the main causes of iron deficiency anaemia.
- Acquired decreased iron absorption has recently been recognized in patients with unexplained or refractory IDA.
- Celiac disease, autoimmune atrophic gastritis, and *H. pylori* infection are increasingly diagnosed in such patients.
- In some cases, *H. pylori* may be directly implicated in the genesis of autoimmune gastritis.
- We strongly recommend a diagnostic work-up for these conditions in case of acquired refractory or obscure IDA.
Causes of Iron Deficiency Anaemia

- Blood loss
- Limited supply (poor diet)
- Increased requirements
- Iron malabsorption

IRIDA

Hereditary

Acquired
DMT1 Deficiency

Severe microcytic anaemia with high transferrin saturation
Severe hypochromia with liver iron overload and normal ferritin levels

<table>
<thead>
<tr>
<th></th>
<th>Father, I-1</th>
<th>Mother, I-2</th>
<th>Birth</th>
<th>Proband, II-1</th>
<th>Normal values (range)</th>
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</thead>
<tbody>
<tr>
<td>Age</td>
<td>35 y</td>
<td>32 y</td>
<td>&lt; 3rd</td>
<td>2 mo</td>
<td>2-3 y</td>
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<tr>
<td>Body weight, percentile</td>
<td>NA</td>
<td>NA</td>
<td>3rd</td>
<td>5th</td>
<td>75 (range)</td>
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<tr>
<td>Hb, g/L</td>
<td>149</td>
<td>128</td>
<td>40</td>
<td>74</td>
<td>130 (120-150)</td>
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<td>MCV, fL</td>
<td>84</td>
<td>79.6</td>
<td>71</td>
<td>75</td>
<td>80</td>
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<tr>
<td>MCH, pg</td>
<td>28.8</td>
<td>27</td>
<td>14</td>
<td>14</td>
<td>14.3 (range)</td>
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<td>Serum iron, μM</td>
<td>14.3</td>
<td>12.9</td>
<td>ND</td>
<td>14</td>
<td>14.3 (range)</td>
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<tr>
<td>Ferritin, μg/L</td>
<td>110</td>
<td>133</td>
<td>ND</td>
<td>255</td>
<td>110</td>
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<td>FEP, μg/g Hb</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>4.7</td>
<td>5.3</td>
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<tr>
<td>Treatment</td>
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<td>None</td>
<td>18 mL PRBCs</td>
<td>25 mL PRBCs</td>
<td>30 mL PRBCs</td>
</tr>
</tbody>
</table>

Top left graphic courtesy of Dr. Achille Iolascon.
The iron cycle

Iron absorption

Enterocyte
- 1–2 mg/day
- Hepcidin-regulated
- Balanced by iron losses (1–2 mg/day)
- Reduced in inflammation
- Increased in iron deficiency

Iron recycling

Macrophage
- 20–30 mg/day
- Hepcidin-regulated
- Balanced by erythroid request
- Reduced in inflammation
- Increased in iron deficiency

CP = ceruloplasmin; DCYTB = duodenal cytochrome B; DMT1 = divalent metal transporter 1; FPN = ferroportin.

Clinical and Laboratory Findings of DMT1 Mutations

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
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<tbody>
<tr>
<td>MCV</td>
<td>45–55 fL</td>
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<tr>
<td>Serum iron</td>
<td>++</td>
</tr>
<tr>
<td>Tf saturation</td>
<td>++</td>
</tr>
<tr>
<td>sTfR</td>
<td>++</td>
</tr>
<tr>
<td>BM sideroblasts</td>
<td>–</td>
</tr>
<tr>
<td>FEP</td>
<td>+</td>
</tr>
<tr>
<td>Liver iron</td>
<td>+++</td>
</tr>
<tr>
<td>Neonatal appearance</td>
<td>+</td>
</tr>
<tr>
<td>Effect oral/IV Fe</td>
<td>–/-</td>
</tr>
<tr>
<td>Serum or urinary hepcidin</td>
<td>–</td>
</tr>
</tbody>
</table>

- **DMT1 is essential in erythropoiesis**
- **DMT1 is not essential for liver iron uptake**
- **DMT1 is not essential for duodenal iron absorption**
  - Alternative pathways?
  - Heme absorption?
- **Increased iron absorption occurs in the presence of iron overload because of low hepcidin levels**
- **Partial response of anemia to erythropoietin treatment**

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Graphic courtesy of Dr. Achille Iolascon.
Genetic defects of iron absorption

IRIDA = iron refractory-iron deficiency anemia (OMIM #206200)

Autosomal recessive

Caused by inappropriately high hepcidin production
Hepcidin downregulation: multiple pathways

Hypoxia, iron deficiency
- (Epo)
- s-HJV
- BMP6
- TWSG1
- GDF15
- Erythroferrone

Erythropoietic expansion
- BMP6
- TMPRSS6
- TMPRSS6 (Epo)
- Erythroferrone

HIF-1α

HFE

TFR2

HJV

BMPR

s-HJV

HRE

HIF-1α
Hepcidin regulation in Iron Deficiency and deregulation in IRIDA

Iron Deficiency

- ↓ HAMP
- ↑ serum iron
- ↓ serum iron
- HAMP
- Normal Erythropoiesis

IRIDA

- ↑ HAMP
- ↓ serum iron
- ↑ serum iron
- ↓ serum iron
- HAMP
- Iron Deficiency
## Hematological parameters of the probands

<table>
<thead>
<tr>
<th>n.</th>
<th>sex</th>
<th>age</th>
<th>Hb</th>
<th>MCV</th>
<th>% TF saturation</th>
<th>Serum Ft</th>
<th>Serum hepc</th>
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<tbody>
<tr>
<td>A</td>
<td>m</td>
<td>6</td>
<td>8.8</td>
<td>58</td>
<td>2</td>
<td>50</td>
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</tr>
<tr>
<td>B</td>
<td>f</td>
<td>13m</td>
<td>9.2</td>
<td>65</td>
<td>10</td>
<td>37</td>
<td>↑</td>
</tr>
<tr>
<td>C</td>
<td>m</td>
<td>17m</td>
<td>7.0</td>
<td>49</td>
<td>5</td>
<td>40</td>
<td>↑</td>
</tr>
<tr>
<td>D</td>
<td>f</td>
<td>11</td>
<td>8.2</td>
<td>56</td>
<td>3</td>
<td></td>
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<tr>
<td>E</td>
<td>m</td>
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<td>27</td>
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<tr>
<td>F</td>
<td>f</td>
<td>3</td>
<td>9.7</td>
<td>61</td>
<td>4</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>G</td>
<td>m</td>
<td>15m</td>
<td>7.9</td>
<td>53</td>
<td>2</td>
<td>59</td>
<td>↑</td>
</tr>
</tbody>
</table>

*(Finberg et al, Nature Genetics 2008;40:569-71)*
Diseases/condition frequently associated with anaemia and/or ID

- Solid tumours and haematologic malignancies
  - ~40% anaemic, 32-60% iron-deficient

- Chronic kidney disease (CKD)\(^4,5\)
  - 27-76% anaemic, 58-73% iron-deficient

- Inflammatory bowel disease (IBD)\(^6\)
  - 6-74% anaemic, 36-90% iron-deficient

- Gastrointestinal disorders (GI)\(^7,8\)
  - 10% (angiodysplasia) to 66% (coeliac disease) anaemic

- Chronic heart failure (CHF)\(^9,10\)
  - 9-79% anaemic, 43% iron-deficient

- Women’s Health conditions\(^11-13\)
  - Heavy menstrual bleeding (HMB): 20% anaemic
  - Pregnancy and postpartum: global 42% anaemic, Europe 25% anaemic
  - Non-anaemic, non-pregnant, premenopausal women: 4-33% iron-deficient

- Special populations (elderly, children)\(^14,15\)
  - Elderly: 3-61% anaemic, Children: 4-7% iron-deficient (US)

6. Kulnigg. Aliment Pharmacol Ther 2006;24,1507;
15. CDC. MMWR 2002;51:897.
Clinical consequences of anaemia and of ID(A)

Central nervous system
- Fatigue
- Dizziness
- Depression
- Impaired cognitive function

Gastrointestinal system
- Anorexia
- Nausea

Vascular system
- Low skin temperature
- Pallor of skin

Immune system
- Impaired T-cell and macrophage function

Cardio-respiratory system
- Exertion dyspnoea
- Tachycardia, palpitations
- Hypertrophy
- Risk of cardiac failure

Genital tract
- Menstruation problems
- Loss of libido

Importance of iron for functioning and survival across all levels of complexity of living structures

Iron is critical for optimal functioning and survival of living structures:

- Mitochondria dysfunction
- Deranged activity of enzymes
- Abnormal transport and structural proteins
- Apoptosis

Iron deficiency results in:

- Tissue remodelling
- Impaired organ efficacy

- Impaired exercise capacity
- Reduced work efficacy
- Impaired cognitive performance and behaviour
- Increased morbidity and mortality

Defective energy metabolism

ID is associated with reduced exercise capacity in heart failure (HF) patients

- Iron deficiency defined as serum ferritin <100 μg/L, or serum ferritin 100–300 μg/L with TSAT <20%
- Anaemia defined as haemoglobin level <12 g/dL in women and <13 g/dL in men
- Iron deficiency was present in 35% of patients


TSAT, transferrin saturation
ID is associated with reduced exercise capacity in HF patients

- Iron deficiency defined as serum ferritin <100 μg/L, or serum ferritin 100–300 μg/L with TSAT <20%
- Anaemia defined as haemoglobin level <12 g/dL in women and <13 g/dL in men

**Peak oxygen consumption**

- Iron deficient: 11.4 mL/min/kg
- Iron replete: 14.9 mL/min/kg

**Relationship between ventilation and VCO₂**

- Iron deficient: 54.8 VE/VCO₂ slope
- Iron replete: 44.1 VE/VCO₂ slope

$P=0.03$ for peak VO₂, $P=0.02$ for VE/VCO₂ slope.
Summary and conclusions

- Iron is an essential nutrient
- ID deficiency is prevalent
- ID presents with a broad spectrum of clinical signs and symptoms
- ID has a considerable impact on patients’ life
- Diagnosis is based on haematological parameters, which have different cut-off values depending on the disease
Anemia Work up and Differential Diagnosis

Examination of CBC and peripheral blood smear

- **MCV < 80**
  - Microcytic anemia
    - Serum iron studies
      - Low iron and ferritin with high TIBC
        - Iron deficiency anemia
      - Mentzer index (MCV/RBC) < 13
        - Thalassemia
  - Anemia of chronic disease

- **MCV 80-100**
  - Normocytic anemia
    - Reticulocyte count
      - <2% (hypoproliferative)
      - >2% (hyperproliferative)
        - Leukemias
        - Aplastic anemia
        - Pure red cell aplasia
        - Hemorrhage

- **MCV > 100**
  - Macrocytic anemia
    - Megalocytes and segmented neutrophils on peripheral smear
      - Present: megaloblastic
        - Vitamin B12 and/or folate deficiency
        - Drug-induced
      - Absent: non-megaloblastic
        - Alcohol abuse
        - Myelodysplastic syndrome
        - Liver disease
        - Congenital bone marrow failure syndromes