Excellence in Rheumatology

« Anaemia of chronic disease »

Charles Masson, MD
Service de Rhumatologie
CHU Angers
FRANCE

Istanbul, Saturday, 19 February 2011

Anaemia of chronic disease

- **1 Causes of anaemia of chronic diseases**
- **2 Iron,**
- **3 Ferritin, Transferrin, Transferrin saturation, serum iron, Soluble transferrin receptor**
- **4 Role for erythropoïetin**
- **5 IL-6, anaemia of inflammation, hepcidin**
- **6 Diagnosing anaemia of chronic disease**
- **7 Treatment of anaemia of chronic disease**

Causes of anaemia of chronic diseases

1 Acute, subacute, and chronic infections.

2 Cancers : most notably of the kidney and pancreas.

3 Hematological malignancies : Hodgkin lymphoma, non-Hodgkin lymphoma, myeloma, Waldenström's macroglobulinemia.

4 Chronic renal failure, chronic hemodialysis.

5 Chronic inflammatory bowel diseases.

7 **Systemic diseases** in the field of the rheumatology : rheumatoid arthritis (rheumatoid anaemia), spondylarthropathies, systemic lupus erythematosus, vasculitides (Horton's disease, polyangiitis), autoinflammatory diseases, adult onset Still's disease.

Other causes of anaemia

- ✓Folates deficiency anaemia or vitamin B12 deficiency.
- ✓Anaemia related to myelodysplastic syndromes
- ✓Anaemia induced by medications such as methotrexate (usually counteracted), salazopyrine, leflunomide, NSAIDS, aspirin.
- ✓Hemolytic anaemia.
- ✓**Iron deficiency anaemia** due to malabsorption or more often **iron loss**.

Anaemia of chronic disease

- 1 Causes of anaemia of chronic diseases
- **2 Iron,**
- 3 Ferritin, Transferrin, Transferrin saturation, serum iron, Soluble transferrin receptor
- 4 Role for erythropoïetin
- 5 IL-6, anaemia of inflammation, hepcidin
- 6 Diagnosing anaemia of chronic disease
- 7 Treatment of anaemia of chronic disease

Iron (I)

- ✓ Iron continuously cycles within the body from the storage sites (liver, spleen) to the marrow or various intracellular compartments and back.
- ✓ Iron is stored in soluble form in ferritin and insoluble form within cells in lactoferrin and hemosiderin.
- ✓ Transferrin and membrane transferrin receptor are require for iron uptake.
- ✓ **The human body has no mechanism for eliminating excess iron.**
- ✓ **Iron is required for the growth of infectious agents.**

Iron (II)

- ✓ Iron amount of an adult : about 4 to 5 g.
- ✓ **Erythrocytes production : 200 billion *per day***.
- ✓ Iron with hemoglobin in the circulating erythrocytes : 2.5 g.
- ✓ The marrow erythroblasts capture 28 mg of iron per day on average to produce hemoglobin.
- ✓ The macrophages continuously phagocytize and catabolize senescent erythrocytes (**erythrophagocytosis**) and the 25 to 30 mg of iron thus released from heme meets the needs of the bone marrow for erythropoiesis.
- ✓ **Inevitable iron losses about 1-2 mg/day** due to epithelial cell shedding (intestinal, skin) are counterbalanced by the intestinal absorption of dietary iron by mature enterocytes : about 1-2 mg/day of iron are absorbed each day for a mean iron intake of 13-18 mg/day
- ✓ Iron losses due to the menstruations : 25 mg/cycle

Anaemia of chronic disease

- 1 Causes of anaemia of chronic diseases
- 2 Iron,
- **3 Ferritin, Transferrin, Transferrin saturation, serum iron, Soluble transferrin receptor**
- 4 Role for erythropoïetin
- 5 IL-6, anaemia of inflammation, hepcidin
- 6 Diagnosing anaemia of chronic disease
- 7 Treatment of anaemia of chronic disease

Ferritin (I)

- ✓ **Ferritin stores iron in an accessible form** : plasma ferritin is a source of readily available iron.
- ✓ Iron bound to ferritin is not toxic.
- ✓ Ferritin is an alpha2 globulin, glycosylated or nonglycosylated, composed of 24 sub-units, which are either heart type (H) or liver type (L).
- ✓ Apoferritin has an accessible central cavity that contains a core of ferric microcrystals.
- ✓ **Each ferritin molecule can store up to 4500 atoms of iron**.
- ✓ Nonglycosylated ferritin (60-80%) comes from parenchymal cells. Its elevation of plasma levels indicates cell lysis.

Ferritin (II)

✓The total amount of ferritin is 1 g in men and 0.4 g in women.
✓The normal plasma ferritin range is in men 30-300 ng/L and 20-200 ng/L in women.
✓Plasma ferritin is best assayed after several days without iron supplementation.
✓Intracellular ferritin contains about 0.5 g of readily available iron.
✓**Plasma ferritin levels in case of inflammatory anaemia are elevated.**
✓**Plasma ferritin values lower than 12-16 ng/L are diagnostic for iron defiency.**

Transferrin (I)

- Transferrin is a plasma beta1globulin, that is produced by the liver, and has a half-life of 8 days.
- Transferrin has a molecular weight of 75 to 80 kDa, with a single protein chain of 679 amino acids, two binding sites for trivalent iron, and two glycan chains.
- **Transferrin may bind one or two iron atoms.**
- **Transferrin not bound to iron is known as apotransferrin.**
- The plasma transferrin level in adults is usually between 1.8 to 3.2 g/L.
- Transferrin is produced chiefly in the liver (16 mg/kg/day).
- To bind to transferrin, iron must be first be oxydized to ferric (trivalent iron), a reaction catalyzed by **hephaestin**, a protein that shares 50% homology with ceruloplasmin (copper-dependent oxidases).

Transferrin (II)

✓**Plasma transferrin levels decrease in the events of**
- **systemic diseases with inflammation.**
- cancers, infections, malnutrition, protein wasting,
- exogenous androgen exposure,

✓**Causes of plasma transferrin levels elevations :**
- iron deficiency,
- increased estrogen impregnation, e.g. during pregnancy, hormone replacement therapy, or oral contraception, although low and ultra-low dose pills have a smaller effect,
- use of thiazide diuretics,
- severe hypogonadism in males.

Transferrin saturation, serum iron levels

- ✓ Transferrin saturation : ratio of serum iron (micromoles/L) over the total iron binding capacity (micromoles/L).
- ✓ Usual values are 20-40% in males, and 15-35% in females.
- ✓ **Transferrin saturation is decreased in both iron deficiency anaemia and anaemia of chronic disease.**
- ✓ Values greater than 45% indicate iron overload.
- ✓ Serum iron levels fluctuate across the 24-hour cycle, whereas transferrin levels do not.
- ✓ **Serum iron is low in iron deficiency anaemia and anaemia of chronic disease and also diminished during menstruations.**
- ✓ Serum iron determination serves to compute transferrin saturation and should be performed in the fasting state, 24-48 hours after stopping any iron supplements (8 days after a blood transfusion or intravenous iron administration).

Bone marrow function of the transferrin receptor

- ✓ The iron used in the bone marrow for erythropoiesis is transferred from the macrophages to the erythroblasts via their transferrin membrane receptors. Only iron bound to transferrin can be used by the erythroblasts.
- ✓ Marrow erythropoietic precursors acquire iron as iron-transferrin complexes via the large number of transferrin receptors at their surfaces.
- ✓ The iron is then exported to the cytosol, and most of it enters the mitochondria, where it is inserted into protoporphyrin IX to form heme.
- ✓ The heme thus formed is exported to the cytosol, where it becomes associated with globin chains.

Soluble transferrin receptor (sTfR)

- ✓ All cells except mature erythrocytes express the transferrin receptor on their surface membrane, but 80% of the receptor molecules in adults are located on the bone marrow erythroblast precursors.
- ✓ This membrane transferrin receptor is a glycoprotein composed of 760 amino acids with two monomers bound by two disulfid bonds to produce a 190 kDa molecule.
- ✓ **The soluble transferrin receptor is the monomeric form of the transferrin membrane receptor.**
- ✓ This soluble transferrin receptor is a marker for erythropoietic activity and tissus iron deficiency.
- ✓ Iron deficiency promptly induces a sharp increase in transferrin receptor production.
- ✓ The plasma sTfR levels is proportional to the amount of transferrin at the surface of hematopoietic cells and increases in proportion to the severity of iron deficiency.
- ✓ **Systemic inflammation does not affect the sTfR levels.**

Study in rheumatoid arthritis of the bone marrow transferrin receptor

- ✓ Bone marrow biopsies were performed in 29 patients with rheumatoid arthritis and anaemia, six patients with iron deficiency anaemia, and nine healthy volunteers.
- ✓ High purity erythroblast fractions were obtained.
- ✓ Transferrin receptor expression at the erythroblast surface was assessed using iodine 135-labeled transferrin and iron 59-labeled transferrin.
- ✓ The rheumatoid arthritis patients were classified as having rheumatoid anaemia or iron deficiency anaemia based on the bone marrow iron content.
- ✓ In rheumatoid anaemia, iron uptake by erythroblast transferrin was increased, although the number of transferrin receptors on the erythroblast surface was not.
- ✓ In iron deficiency anaemia, both iron uptake and the number of surface transferrin receptors were increased, and serum soluble transferrin receptor was elevated.

Anaemia of chronic disease

- 1 Causes of anaemia of chronic diseases
- 2 Iron,
- 3 Ferritin, Transferrin, Transferrin saturation, serum iron, Soluble transferrin receptor
- **4 Role for erythropoïetin**
- 5 IL-6, anaemia of inflammation, hepcidin
- 6 Diagnosing anaemia of chronic disease
- 7 Treatment of anaemia of chronic disease

Role for erythropoïetin (I)

- ✓ Erythropoeitin is a 30,4 kDa glycoprotein composed of 165 amino acids.
- ✓ Erythropoeitin inhibits erythroid progenitor apoptosis and induces clonal proliferation of normoblasts.
- ✓ **The kidneys produce 90 to 95% of the erythropoietin production in adults.**
- ✓ **Hypoxia activates erythropoietin production.**
- ✓ Erythropoietin was first purified in 1977, and the gene was cloned in 1983. The first clinical studies were started in 1986. The introduction of erythropoietin therapy has virtually eliminated the need for blood transfusion in chronic hemodialysis patients.

Role for erythropoïetin (II)

In anaemia of chronic disease as rheumatoid arthritis :

- the high proinflammatory cytokine levels usually inhibit the production of erythropoietin mRNA to some extent, leading to a decrease in endogenous serum erythropoietin.
- An increased production of suppressor of cytokine signaling/cytokine inducible SH2 protein may lead to a resistance to the effects of erythropoieitin (« blunted erythropoietin response »).
- But other pathological mechanisms are described in erythropoiesis, especially early hematopoiesis. TNFalpha and interferon gamma inhibit erythroid colony formation in vitro.

Anaemia of chronic disease

- 1 Causes of anaemia of chronic diseases
- 2 Iron,
- 3 Ferritin, Transferrin, Transferrin saturation, serum iron, Soluble transferrin receptor
- 4 Role for erythropoïetin
- **5 IL-6, anaemia of inflammation, hepcidin**
- 6 Diagnosing anaemia of chronic disease
- 7 Treatment of anaemia of chronic disease

IL-6 and anaemia of chronic disease

- ✓ IL-6, a 21kDa polypeptide composed of 212 amino acids arranged in four alpha chains, is a proinflammatory cytokin produced by numerous cell types.
- ✓ **IL-6 is closely involved in the development of inflammatory anaemia.**
- ✓ Thus in patients with advanced ovarian cancer, the severity of anaemia correlates with the degree of IL-6 elevation. The IL-6 together with cancer stage predicts the severity of anemia.
- ✓ The infusion of human recombinant IL-6 leads to two kinds of anaemia : dilution anaemia and inflammatory anaemia.
- ✓ Within 24-48 hours, IL-6 downregulates transferrin expression and upregulates ferritin expression, wheras serum iron levels drop within 12 hours.
- ✓ In non human primates, IL-6 diminishes the proportion of nucleated cells in the bone marrow and decreases serum iron levels. These effects can be corrected by administering an IL-6 antagonist.

Discovery of the link between iron and IL-6 : hepcidin

- ✓ Hepcidin lowers serum iron levels by acting as a gatekeeper for membrane iron transport.
- ✓ Hepcidin is a circulating peptide with a key role in iron homeostasis.
- ✓ The « initial » role of hepcidin is the development of hypoferremia within hours of infection as part of the acute phase and the innate immune responses.
- ✓ Low serum iron concentration in the host are thought to limit the proliferation of invading pathogens.

hepcidin comes from pro-hepcidin

- ✓ Hepcidin comes from pro-hepcidin, an 84-amino acid peptide, produced by the liver, as well as in the macrophages, kidney cells, and adipocytes.
- ✓ Pro-hepcidin is cleaved by the proprotein convertase furin, which releases hepcidin, the mature peptide containing only 20 to 25 amino acids with eight cystein residues linked by four disulfide bonds.
- ✓ Hepcidin is eliminated through the urine.
- ✓ Hepcidin expression in the liver is dependent on the protein hemojuvenil, co-receptor of bone morphogenetic proteins, that activates hepcidin gene transcription via a Smad4-dependent pathway.

Hepcidin : gatekeeper for membrane iron transport

- ✓ Hepcidin prevents iron from exiting cells, most notably enterocytes, macrophages, and hepatocytes, thereby decreasing iron levels in the bloodstream.
- ✓ Hepcidin inhibits both intestinal iron absorption and the release of iron stored in macrophages and hepatocytes.
- ✓ Hepcidin acts by binding to ferroportin, a protein that exports ferric iron from cells. Hepcidin causes the internalization, ubiquitination, and lysosomal degradation of ferroportin.

Hepcidin and interleukine-6

- ✓ Hepcidin gene transcription, most notably in the hepatocytes, is increased during inflammation, via IL-6.
- ✓ IL-6 rapidly increases the production of hepcidin.
- ✓ When human hepatocytes are exposed to a cytokine panel, only IL-6 upregulates the production of hepcidin mRNA.
- ✓ In healthy human volunteers, IL-6 infusion increases the release of hepcidin 7.5 fold, and decreased the ferremia.
- ✓ *In vitro* IL-6 induces the production of hepcidin by human hepatoma cells.
- ✓ Il-6 knockout mice have subnormal hepcidin levels and high serum iron levels.
- ✓ In wild type mice, turpentine exposure to activate the inflammatory response markedly upregulates hepatic hepcidin expression.

Recently several hepcidin assays have become available

✓Hepcidin tends to be correlated most closely with serum ferritin, reflecting its regulation by both inflammation and iron stores.

✓An ELISA for the 83-amino acid pro-hepcidin is available, but this form is not bioactive, and does not correlated with the 25 amino-acid, bioactive form of hepcidin in the serum.

✓**Variations of the production of hepcidin :**

- In iron deficiency and other situations associated with increased erythropoiesis (e.g. hypoxia, bleeding, hemolysis, dyserythropoiesis), hepcidin synthesis is suppressed.
- Inflammation upregulates hepcidin production via IL-6.

Anaemia of chronic disease

- 1 Causes of anaemia of chronic diseases
- 2 Iron,
- 3 Ferritin, Transferrin, Transferrin saturation, serum iron, Soluble transferrin receptor
- 4 Role for erythropoïetin
- 5 IL-6, anaemia of inflammation, hepcidin
- **6 Diagnosing anaemia of chronic disease**
- 7 Treatment of anaemia of chronic disease

Diagnosing anaemia of chronic disease

✓The World Health Organization defines anaemia as hemoglobin < 13 g/dl in men and < 12 g/dl in women.
✓Mean cell volume, and mean cell hemoglobin are normal, but can drop as the disease persists.
✓Levels of serum ferritin are normal or elevated.
✓Levels of transferrinemia are normal or low.
✓Transferrin saturation is low.
✓Soluble transferrin receptor levels are not increased.
✓The ratio soluble transferrin receptor/log serum ferritin is less than 0.8.
✓The bone marrow macrophages iron stores are normal or elevated (Prussian blue iron staining).
✓In iron deficiency anaemia : serum ferritin is low, transferrin is normal or elevated transferrin, soluble transferrin levels increased. Many causes (comorbidities) are possible.

Anaemia of chronic disease

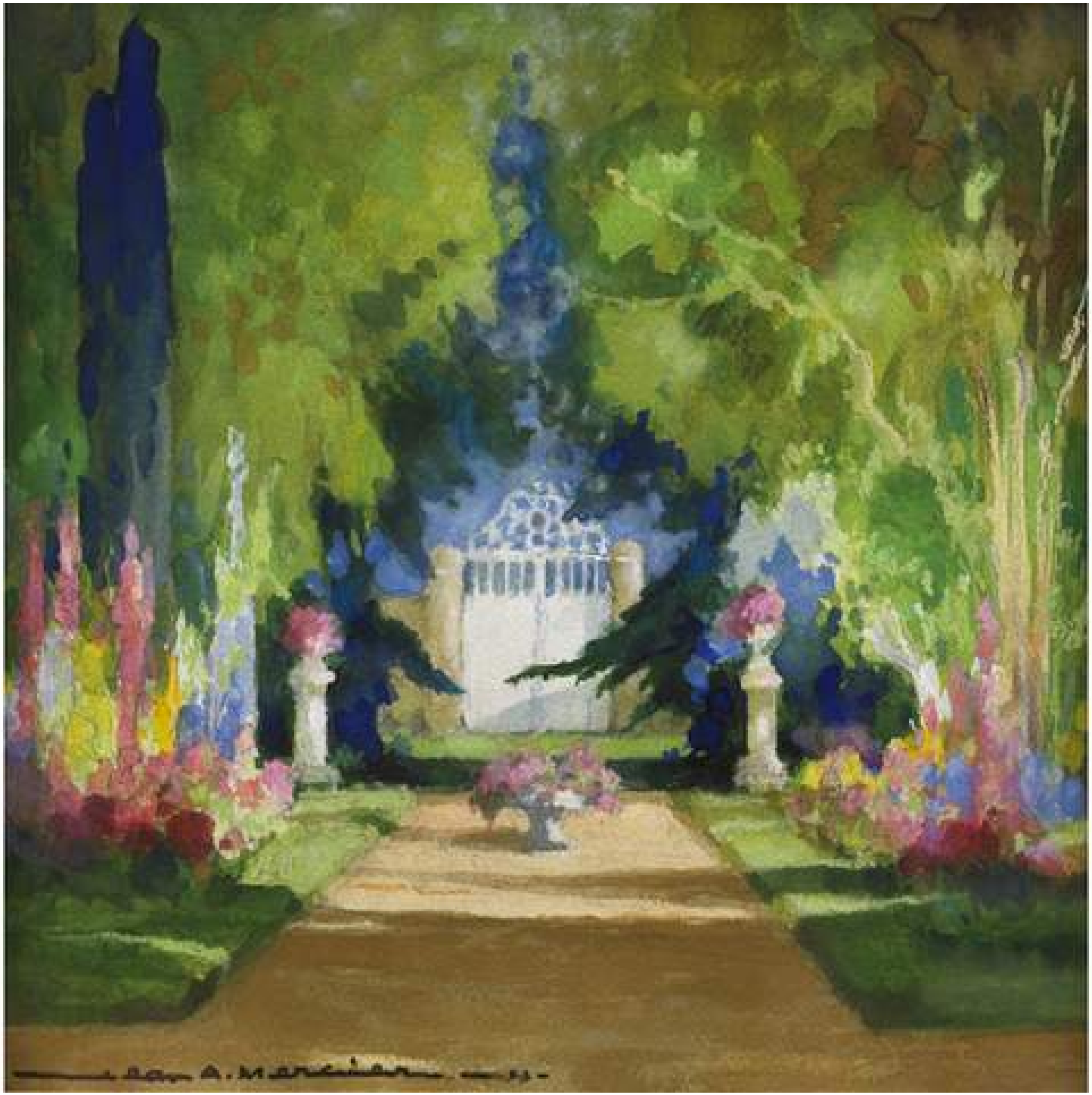
- 1 Causes of anaemia of chronic diseases
- 2 Iron,
- 3 Ferritin, Transferrin, Transferrin saturation, serum iron, Soluble transferrin receptor
- 4 Role for erythropoïetin
- 5 IL-6, anaemia of inflammation, hepcidin
- 6 Diagnosing anaemia of chronic disease
- **7 Treatment of anaemia of chronic disease**

Treatment of anaemia of chronic disease

- ✓ **The primary goal for the treatment of anaemia of chronic disease is to treat and cure the underlying source of inflammation.**
- ✓ Transfusions of red blood cell packs are very rarely appropriate for the treatment of anaemia of chronic disease.
- ✓ Oral iron supplementation is required in patients with documented iron deficiency independently from the chronic disease.
- ✓ In patients scheduled for surgery, intravenous colloidal iron once weekly (at a dose of 100 to 200 mg) for a few weeks may deserve consideration.
- ✓ The prevention of digestive blood loss is always in mind.
- ✓ Classicaly, in rheumatoid arthritis, oral iron supplementation for rheumatoid anaemia was believed to carry a risk of rheumatoid arthritis flare. But no flare occurred of a small number of rheumatoid arthritis patients given intravenous iron.

Pharmacological agents that activates erythropoiesis

- ✓ **Recombinant human erythropoietin :**
- Epoeitin alpha (Epogen, Procrit/Eprex) intravenous half- life 8.5 hours.
- EPO beta (NeoRecormon) intravenous half-life 8.5 hours.
- Darbepoetin alpha (Aranesp) intravenous half-life 25.3 hours.
- ✓ **Induces continuous activation of erythropoietin receptors** : Epoetin beta-methoxy polyethylene glycol (CERA), half-life 130 hours.
- ✓ **Activates erythropoietin receptors** : hematide, a synthetic erythropoietin receptor agonist.



Hepcidin values and diseases

- In a cross-sectional study, serum pro-hepcidin levels were significantly higher in 72 patients with rheumatoid arthritis than in 28 patients with systemic lupus erythematosus.
- Another study found that serum prohepcidin was significantly lower in 30 patients with pure iron deficiency than in 30 patients suffering from rheumatoid arthritis with non iron deficient anaemia or 20 healthy controls. The levels of the soluble transferrin receptor were higher in iron deficient anaemia.
- Serum hepcidin levels were significantly higher in 19 patients with rheumatoid anaemia than in 12 patients with iron deficiency anaemia or 14 healthy controls.

Causes of iron deficiency anaemia

1 Drugs : nonsteroidal antiinflammatory drugs, antiplatelet drugs, and glucocorticoids. Esophagitis, gastric or duodenal ulcers, bowel lesions.

2 Gynecological disorders : menstruations, myoma, endometrial cancer.

3 Digestive system disorders : tumors, inflammation, or systemic disease affecting the eosophagus, stomach, duodenum, small bowel, colon, or rectum.

4 Malabsorptions, celiac disease.

5 Kidney diseases.

6 **Special situations** :

Heyde syndrome : angiodysplasia, aortic stenosis, and acquired

Von Willebrandt factor abnormalities.

Rendu-Osler disease. Hemosiderosis.

Factitious disorder : Lasthenie de Ferjol syndrome.

Iron deficiency anaemia of unexplained cause : chance of identification of a digestive lesion

- ✓ Among patients with unexplained iron deficiency, about half have lesions identified by endoscopic examination of the upper gastrointestinal tract and colon.
- ✓ When no lesion is found, repeating both endoscopic examinations identifies a lesion in only 6 per cent of cases.
- ✓ When a tumor or other small bowel lesion is suspected, computed tomography enteroclysis may be indicated, followed by video capsule enteroscopy (if there is no stenosis).
- ✓ Enteroscopy may be (at last) performed intraoperatively through an incision in the gut wall.

Erythropoietin in anaemia of chronic disease

- ✓ Recommendations are available for three causes of anaemia of chronic disease : cancer, chronic kidney failure and chronic bowel disease. Colleges of Rheumatology have not issued specific recommendations for erythropoietin use.
- ✓ The goal of erythropoetin therapy is usually to increase hemoglobin level to 11 or 12 g/dl.
- ✓ Side effects of erythropoietin (arterial hypertension, vascular thrombosis) occurrend when the hemoglobin level increases by more than 1 g every two weeks.
- ✓ In patients with persistent anaemia despite specific treatment, erythropoietin therapy may be considered if the serum erythropoietin levels are low given the degree of anaemia (lower than 500 mU/ml or even 100 mU/ml). In this situation, intravenous iron supplementation may also deserve consideration.
- ✓ In the trial « Dialysis patients' Response to IV iron and with elevated ferritin », intravenous ferric gluconate improved hemoglobin production in the patients with a high serum ferritin levels (500-1200 ng/ml) compared to the controls.

IRIDA : Iron Refractory Iron Deficiency Anaemia

- Inactivating mutations in the gene for matriptase-2, a protein involved in hepcidin regulation.
- High hepcidin levels.
- Low serum iron, normal-to-low serum ferritin.
- Microcytic, hypochromic anaemia.
- No response to oral iron supplementation.
- Response to intravenous iron supplementation.

Bone Marrow iron

- ✓ Perls' stain for iron is performed in selected clinical situations or in research projects with the consent of the patient.
- ✓ With Perls' stain, iron in hemosiderin, mitochondria, and Papenheimer bodies is stained blue-green, whereas neither ferritin nor hemoglobin is stained.
- ✓ The normal or increased iron stores seen in bone marrow macrophages in rheumatoid anaemia contrast with the iron depletion of macrophages and erythroblasts in iron defiency anemia.
- ✓ Presence of sideroblasts, which are erythroblasts containing iron granules, suggests primary or secondary sideroblastic anaemia.

Anaemia of inflammation and early hematopoiesis

- ✓ TNF-alpha and TGF-beta inhibit the proliferation of murine progenitors and self-renewal of pluripotent stem cells.
- ✓ TNF-alpha, interferon gamma, and TGF-beta synergistically activate human CD34+ cell apoptosis vis the fas/Fas-ligand system.
- ✓ TNF-alpha can induce cellular senescence.
- ✓ In rheumatoid arthritis, in a study comparing 40 patients to 24 age- and sex-matched controls, all of whom underwent bone biopsy, the rheumatoid arthritis patients had fewer erythroid progenitors and precursors than did the controls.