

## NEW KNOWLEDGES ON RARE ANAEMIAS

### REPORT FROM THE EUROPEAN IRON CLUB MEETING

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*The European Iron Club (EIC) Meeting was held in Barcelona September 28-29 in the beautiful venue of the historical Hospital "de la Santa Creu I San Pau". Although the EIC Meeting is traditionally aimed at updating basic and clinical advances in all fields of iron metabolism, due to the tight connections between iron and red cells, some of the results presented at the Meeting are potentially of interest to ENERCA members and are here summarized.*

*Anemia of chronic diseases (ACD) is not a rare anemia, but has a complex pathogenesis that is only partially unravelled by recent studies. A lecture on this topic was given by Gunter Weiss - Innsbruck.*

#### **Anemia of chronic diseases**

Anemia of chronic disorders (ACD) is a rather common cause of anemia usually of moderate degree, underlying chronic infections, inflammation and cancer. ACD is traditionally linked to blunted erythropoietin production, impaired proliferation of erythroid progenitors and abnormalities of iron metabolism. Recent advances indicate that ACD is an immunity driven disorder due to the activation of a network of pro and anti-inflammatory cytokines, which play the major role in depressing erythropoietin response and erythroid cell proliferation and cooperate with the liver peptide hepcidin to alter iron homeostasis. Hepcidin is a recently identified liver peptide with a key regulatory function in duodenal iron absorption and macrophage iron release, through binding and regulation of the activity of the cellular iron exporter ferroportin. Binding of hepcidin causes internalization and lysosomal degradation of ferroportin, thus reducing iron export to circulating transferrin both from duodenal cells and macrophages. Hepcidin is an acute phase protein and, as such, is strikingly increased in inflammation (both acute and chronic) in response to increased IL-6 and other cytokines. Its increase causes macrophage iron sequestration and blocks intestinal iron absorption. This translates on one side in increased iron stores and increased serum ferritin and on the other side in reduced transferrin saturation, decreased iron supply to the marrow and iron restricted erythropoiesis. Diagnosis of ACD at present relies on a test combination: iron abnormalities are the hallmark of the condition: decreased serum iron concentration, low transferrin saturation in the presence of normal/high serum ferritin. However, in some patients chronic bleeding may cause absolute iron deficiency, challenging the correct diagnosis, because it is difficult to recognize iron deficiency in the context of ACD. Since ferritin is low in iron deficiency and normal or decreased in iron deficiency associated with ACD and soluble transferrin receptor (sTFR) is high in iron deficiency and normal or high when both conditions are present, the ratio sTFR/logferritin was proposed as a differential test. Low ratios (<1) characterize ACD, high ratios (> 2) are present in iron deficiency or in associated conditions. Problems still concern the availability of the sTFR test and its standardization. Therapeutic options in ACD are variable and include: recombinant erythropoietin, iron, blood transfusions, according to patient disorder and degree of anemia. Basic to all forms is the treatment of the underlying disorder, if possible, since regression of the inflammation is associated with a full recovery from ACD. However, other therapeutic measures may differ according to the iron status. When planning the patient treatment it should be considered that the best therapeutic regimen must include the evaluation of the quality of life, the cardiac performance and the underlying disorder.

*Liver is the main but not the only organ that may produce hepcidin.*

## **The regulation and role of macrophage hepcidin during inflammation and in ACD patients**

*Contributed by Theurl I - Innsbruck*

This selected oral presentation by the Gunter Weiss group showed that hepcidin in inflammation is produced not only by the liver but also by the inflammatory cells, leukocytes and monocytes, in a toll receptor (TLR-4)-dependent and in an iron-independent way. Ferroportin internalization and degradation occurs in monocytes that overexpress hepcidin. In cell culture and in experimental animals monocyte hepcidin responds to LPS and IL6, as liver hepcidin. On a whole the data suggest that an autocrine pathway (monocyte hepcidin increase and monocyte ferroportin degradation) contributes to iron sequestration in inflammation.

*Hepcidin was indeed a central topic of the Meeting, discussed in at least 3 of the 4 lectures. ACD is an example of anemia associated with abnormally increased hepcidin. On an opposite side decreased hepcidin synthesis has a role in causing iron overload in thalassemia.*

## **Hepatic hepcidin mRNA is decreased in thalassemia: a study of 23 patients**

*Contributed by Camberlein et al - Rennes, France*

On an opposite side decreased hepcidin synthesis was documented in a series of iron loaded thalassemic patients. The study concerns 23 thalassemic patients, 10 with thalassemia major and 13 with thalassemia intermedia. Dosing liver hepcidin mRNA, obtained by liver biopsies, using real time quantitative PCR the authors showed that liver hepcidin mRNA expression was significantly lower in thalassemia intermedia patients than in thalassemia major.. Thalassemia intermedia patients had higher degrees of anemia than thalassemia major, whose anemia was corrected by chronic blood transfusions. Positive correlation was observed between hepcidin mRNA and Hb levels, but not with hepcidin and liver iron concentration. The conclusion was that in iron loading anemias the predominant effect on hepcidin expression is exerted by the erythropoietic drive rather than by the iron stores. This study complements a series of recent reports that, along the same line, indicate that iron overload in transfusion-independent thalassemia develops because of hepcidin suppression, as in genetic hemochromatosis, and is related to the erythropoietic expansion. The mechanism underlying this control remains to be understood.

*Sideroblastic anemia is characterized by defective mitochondrial iron utilization and mitochondrial iron accumulation, as revealed by ringed sideroblasts. The following report is courtesy of Alison May, who presented the paper at the European Iron Club (EIC) Meeting.*

## **Successes and difficulties of diagnosing the causes of sideroblastic anaemia**

*Contributed by Alison May, PhD, Cardiff University School of Medicine, UK*

Another anaemia associated with iron loading is sideroblastic anaemia, a group of disorders heterogeneous in nature and cause, characterized by different patterns of inheritance. The anaemia can occur in isolation or in combination with pathological change in other tissues and organs. Genetic diagnosis is of great importance for patient, family and physician, however, a review of 66 anaemic patients with sideroblastic anemia revealed that diagnosis is made in less than half. About one third of all had variations in the X-linked ALA Synthase 2 gene. One quarter of patients with ALA Synthase 2 defects are female with markedly skewed X chromosome inactivation, leading to either small, pale red cells characteristically found in the male patients, when the defect is moderate or, paradoxically, large, well-haemoglobinised red cells when the defect is severe. Proof that a new mutation has a direct role in anaemia often requires detailed and time-consuming studies. However, recent publication of the first 3D structure of bacterial ALA Synthase ([Astner I, EMBO J 2005;24; 3166-77](#)) enables modelling the human enzyme and its variants, facilitating this assessment. The other four known genetic causes of sideroblastic anemia were less common. None were thought likely to have defects in either the thiamine transporter-1 that give rise to a thiamine-responsive megaloblastic anaemia, deafness and diabetes mellitus, or the mitochondrial enzyme pseudo-uridine synthase 1 that cause mitochondrial myopathy and lactic acidosis, both of which are autosomal recessive conditions. One severely anaemic patient had a sporadically-occurring Pearsons syndrome (pancreatic exocrine deficiency and lactic acidosis) caused by a large deletion of mitochondrial DNA. Three members of the same family with cerebellar ataxia had a variation in the structure of the X-linked, mitochondrial,

ABCB7 transporter protein, involved in iron-sulphur clusters transport to the cytoplasm and one young patient carried a splice-site ABCB7 gene variation of uncertain significance. Measurement of red blood cell zinc protoporphyrin and free protoporphyrin was considered a valuable tool to address molecular studies. Patients with ALA Synthase defects had low/normal levels of both, patients with ABCB7 structure defects had mainly increased zinc protoporphyrin and two patients, only one of whom was a compound heterozygote for ferrochelatase defects, had increased free protoporphyrin with a diagnosis of Erythropoietic Protoporphyrin, a condition rarely associated with sideroblastic anaemia. All of these patients had microcytic, hypochromic red cells and porphyrin measurements helped limit candidate genes. Newly-discovered mitochondrial proteins, or old proteins with newly-discovered roles, such as mitochondrial ferritin, frataxin and mitoferrin, may yet be found to have parts to play in sideroblastic anaemia and factors involved in their regulation will emerge and may be additional candidates. Studies of iron and sulphur metabolism in yeast and bacteria have facilitated identification of human counterparts, in particular within the mitochondrion, loss of which causes microcytosis and hypochromia, decrease ALA Synthase 2 activity and haem production but increase mitochondrial iron uptake. Besides the ABCB7 transporter other genes, including the copper transporters that are essential for intracellular iron homeostasis, may play a role in inherited forms of sideroblastic anaemia. Simple tools to probe these pathways need to be designed.