

## CONGENITAL DYSERYTHROPOIETIC ANEMIAS

### NEW OBSERVATIONS, AND PRACTICAL ASPECTS OF DIAGNOSIS AND THERAPY

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The congenital dyserythropoietic anemias (CDAs, ICD-10 D64.4) comprise a group of rare hereditary disorders that are characterized by ineffective erythropoiesis as the predominant mechanism of anemia and by distinct morphological abnormalities of erythroblasts in the bone marrow. Although these disorders were first recognized 40 year ago, there are still many open questions of both molecular genetics, timely and accurate diagnosis and therapeutic decision making. This newsletter reviews the most pertinent genetic, epidemiological and clinical data and provides a forecast to ENERCAs efforts to collect and to analyze data from different European countries, with the goal to improve physicians management of the affected individuals.

The present state of knowledge was recently reviewed in an ESH monograph[7]. There are three entities with well defined phenotypic features, and a variety of so called "aberrant" or "variant" phenotypes (Table 1) . Although the morphological and clinical features were well described more than 40 years ago and repeatedly reviewed, the correct diagnosis is often delayed to adolescence or adulthood, and still in the last decade some cases had already clinical relevant iron overload when the diagnosis was made and appropriate management was instituted. The most frequent erroneous diagnoses are hereditary spherocytosis, unclassified congenital hemolytic anemia, intermediate thalassemia, hereditary hemochromatosis, megaloblastic anemia, or even Gilberts disease, the latter particularly in cases with moderate or borderline anemia and concomitant reduced expression of uridine diphosphate glucuronyl transferase[18]. Out of the definition criteria (Table 2), laboratory data suggesting ineffective erythropoiesis is the most relevant criterion to distinguish CDA from true hemolytic anemia. In practice, this relies on normal or inadequate reticulocyte counts in spite of anemia. In CDA II, however, there is also peripheral hemolysis in addition to ineffective erythropoiesis, and reticulocyte counts may be as high as 5 % in some cases [5; 11].

#### Diagnosis

The primary findings leading to the suspected diagnosis of any type of the CDA are anemia of varying degree and indirect hyperbilirubinemia. In many cases there is moderate splenomegaly with a palpable spleen. However, in non severe cases, splenomegaly may be absent in childhood. The next diagnostic steps are:

1. To ensure congenital anemia and/or hyperbilirubinemia by careful history including ascertainment and review of previous blood counts.
2. To do blood counts on first degree family members. However, in the majority of CDAs, inheritance is autosomal recessive, and due to the small number of offspring in most European families, single cases in one family are the rule rather than the exception.
3. To repeat full blood counts. Mean corpuscular volume (MCV) and Mean Corpuscular Hemoglobin (MCH) may be moderately low in children, but are usually normal in adolescents and adults or increased in CDA type I. Do at least three reticulocyte counts and if they are higher than normal estimate whether the increase is adequate as compared with the degree of anemia.

4. To examine the peripheral blood smear by microscopic analysis. Anisocytosis and poikilocytosis are always present, but not specific. A few microspherocytes may be seen, but hereditary spherocytosis as well as thalassemia will be recognized by an experienced investigator. More specific is basophilic stippling, and Cabot rings may be observed in CDA I. Single erythroblasts are present in about 80% of the cases, and if found, binucleated normoblasts suggest the diagnosis of CDA II.
5. To exclude another form of congenital anemia listed above, taking in consideration the prevalence in the region from which the patients family comes. Thalassemia has to be ruled out in all families from regions with high prevalence such as the Mediterranean countries, and also in all patients with low MCV and MVH. Exclude Vitamin B12 or folate deficiency in patients with high MCV and MVH, particularly if the congenital nature of the disorder is not unequivocal.
6. If the diagnosis of any type of CDA cannot be ruled out by these diagnostic steps, bone marrow examination is mandatory, and may enable not only the diagnosis of CDA, but also the recognition of the type. The diagnosis is much easier in aspirates than in histobiopsies. Typical examples can be found under [http://bildatlas.onkodin.de/bildatlas/content/e1352/e1775/e1872/e2500/e2501/index\\_ger.html](http://bildatlas.onkodin.de/bildatlas/content/e1352/e1775/e1872/e2500/e2501/index_ger.html). Iron stains should be done to exclude hereditary sideroblastic anemia. Since all typical abnormalities may be seen in single cells of any type of erythropoietic hyperplasia, the magnitude of aberrations is relevant, rather than the type of abnormality as such. The percentage of "CDA-erythroblasts" is between 10 and 50 % of all erythroblasts in CDA.

If the diagnosis of a CDA is secured or strongly suggested, further steps are recommended

1. To identify the exact type. Since some therapeutic measures are type-specific, this is of not only of academic, but even more of practical interest. These steps should be made in cooperation with expert centers, that can be found at the ENERCA website ([www.enerca.org](http://www.enerca.org)). In cases of type I or II, bone marrow morphology permits an almost, but not always secure classification. Some cases reported in the literature had to be reclassified at review of the data by experts.
2. If CDA I is suspected, the diagnosis can be supported by sequencing the CDANI gene. Mutations on 7 different exons have been reported, and were not found on a variety of controls [21; 23]. Mutations may be monoallelic in affected individuals. Absence of a relevant mutation does not finally rule out CDA I, because very rare cases were observed with mutation in another, hitherto unrecognized gene [27].
3. If CDA II is suspected, final diagnosis relies on evidence of the specific membrane abnormality. The classical test is the acid-serum-lysis test, first reported by Crookstone [2], giving the disorder the synonym HEMPAS = Hereditary Erythroblastic Multinuclearity with a Positive Acidified Serum test. This test, however, that is a modification of the so called Hams-test formerly used for the diagnosis of PNH, needs experience in serological techniques and the availability of sera that contain natural Anti-Hempas antibody. Recent work of ENERCA members (publication pending) has shown that abnormality of glycosylation of Band 3 membrane glycoproteins demonstrated by SDS-PAGE is reproducible in different specialized laboratories and is highly sensitive and specific for the diagnosis of CDA II. It should become the routine procedure to proof CDA II. Since the CDA II gene has not yet been identified, genetic (molecular?) diagnosis is not yet available. The sporadic type of CDA III is very rare, and utmost caution is recommended to make this diagnosis. Some of the reports from the literature are far from convincing, and one case published as CDA III [20] was identified as CDA II when reviewed by the author of this newsletter. The diagnosis of an aberrant type of CDA needs workup by specialist, and requires special studies including electron microscopy
4. When the diagnosis of a CDA and the identification of the type has been made, further studies are necessary to recognize complications and to decide on therapeutic measures. The most relevant tests are: Abdominal ultrasound for exclusion of gall stones, that are observed in about 80% of patients at follow up and may be observed in children or adolescents. Negative results should be controlled in yearly intervals. Search for anti-human parvovirus B19 antibodies. If no IgG antibodies are found, the patient is at risk for an aplastic crisis [10]

Since folate deficiency is observed more frequently in conditions with hyperplastic erythropoiesis folate levels should be measured. Tests for estimate iron stores. Serum ferritin, serum transferrin and transferrin saturation should be measured and controlled at least in yearly intervals. Soluble transferrin receptor concentration reflects the magnitude of erythroid hyperplasia; it is regularly increased, but as well as in hemolytic anemias or thalassemias, it does not reflect the amount of storage iron. If possible, liver iron should be measured by a noninvasive method such as SQUID or MRI, to have a basic value for later follow up.

## Epidemiology

CDAs were observed from many regions of the world. CDA II has more frequently reported from South Italy, but this may be due to a founder population[12]. Since all types of CDA require bone marrow analysis, many cases probably remain undetected in areas where special studies in patients with chronic anemias are difficult to perform. ENERCA attempts to obtain more complete data from the European countries. Data from the German CDA registry presented at the ENERCA symposium in March 2007 are shown in Figure 1.

## Management of patients with CDA

Pediatricians and hematologists only occasionally see a patient with CDA and therefore should seek the advice of the few specialists interested in research on rare congenital anemias. European ([www.enerca.org](http://www.enerca.org)) and national ([www.bone-marrow-failure-syndromes.de](http://www.bone-marrow-failure-syndromes.de)) networks provide information of specialist centers and give access to new knowledge for both physicians and patients. Decision making depends on age, type of CDA, severity of expression and comorbidity.

Two procedures are effective to improve the chronic anemia: Interferon- $\alpha$  in CDA I and splenectomy in CDA II. Prednisone, folate or cobalamine was tried in many patients without evidence of efficacy, as well as human recombinant erythropoietin in CDA I [24] and CDA II (own observations, unpublished). **Interferon- $\alpha$**  in doses of approximately 10 Million Units per week led to normal hemoglobin concentrations in 10 adult patients with CDA I reported in the literature [19], and the same effect was achieved by 30 - 50  $\mu$ g Pegylated interferon  $\alpha$  2b [4; 13]. When interferon therapy is stopped, hemoglobin level returns to previous values. Erythrokinetic studies demonstrated a striking reduction of the ineffective erythropoiesis, and electron microscopy studies showed a reduction in nuclear structure abnormalities[14]. Low dose interferon is a long-term treatment of CDA-I, and allows a significant decrease in iron overload, that could be interesting? relevant?? even in patients who are only moderately anemic.

In CDA II, **splenectomy** leads to a moderate and sustained increase in hemoglobin concentration and decrease of serum bilirubin levels, as shown in 48 patients from the two registries [5; 11] and in 41 patients published as case reports. If measured, shortened red cell survival normalizes [1], demonstrating that, as in hereditary spherocytosis, abnormal CDA II erythrocytes may survive normally in an asplenic individual. No overwhelming bacterial infections were observed after splenectomy. One case of Budd-Chiari syndrome was reported [26]. Portal vein thrombosis occurred in two patients [6]. Splenectomy does not prevent further iron loading, even in those cases where hemoglobin concentrations becomes nearly normal[5]. This may be explained by the observation that the expansion of the erythroid marrow is more closely correlated to iron loading than the anemia itself, which in CDA II is determined by both ineffective erythropoiesis and shortened red cell survival. The main benefit of splenectomy is abrogation of transfusion requirements in more severe cases, and increase of the hemoglobin concentration to allow regular phlebotomies. In other patients, it is advocated to follow the guidelines for mild cases of hereditary spherocytosis [15]. A current project of ENERCA organized by A. Iolascon collects data on splenectomized patients with CDA II, and attempts to build an evidence based guideline for individual decision making and subsequent surveillance. Splenectomy is not recommended in CDA I, and individual decisions have to be made in variants with transfusion dependency and an enlarged spleen. Most adolescent and adult patients with CDA have only mild or moderate anemia. However, independent of the severity of the anemia, there are clinically relevant consequences in almost all, and complications in a few patients.

The main problem encountered by patients after the first years of life is **iron overloading**, which is also seen in patients without ongoing need for transfusion. The fact that patients with both CDA I and II as well as variant types store iron in a manner similar to those with other chronic states of ineffective erythropoiesis has been known since the early observations[9]and

was confirmed by many case reports. Iron accumulates steadily throughout life especially in the liver (are there studies on cardiac iron?) , with kinetics similar to untreated hereditary hemochromatosis [5] [8]. There is distinct variability among individuals, which is not explained by HFE gene polymorphism [11; 22; 25]. Transfusions contribute to iron overload, and this risk has to be individually weighed against the failure to thrive in infants and children with severe anemia, and against the risk of damage to the mother and the fetus in pregnancy. As mentioned above, even in patients with slight or moderate anemia, ferritin levels should be controlled in at least yearly intervals, because iron overload may approach risk levels at any age. Adequate treatment with regular phlebotomies and /or deferoxamine leads to normal ferritin concentrations, indicating reversal of iron overload in patients with CDA. No data on the substitution of deferoxamine by the oral chelator deferasirox (Exjade®) were published, but single own observations do not suggest disease specific undesired side effects. Since data correlating serum ferritin values to tissue iron are scarce, prospective studies using noninvasive techniques of liver iron determination by SQUID [3] or MRI are required; at present, management of iron overload should follow guidelines for thalassemia [16; 17].

**Allogenic stem cell transplantation** was successful in some children with exceptionally severe anemia [7]. It requires careful decision making by a specialized expert.

Rare complications include **transient aplastic crisis** necessitating red cell transfusions [10], bulky extramedullary hematopoiesis, which may result in risky operations (Fig. 2), and chronic leg ulcers. The latter may severely compromise quality of life in higher age. Since anemia is one of the etiologic factors, they should be considered if decision on anemia treatment is made.

**Table1. Characteristic features of different types of congenital dyserythropoietic anemia**

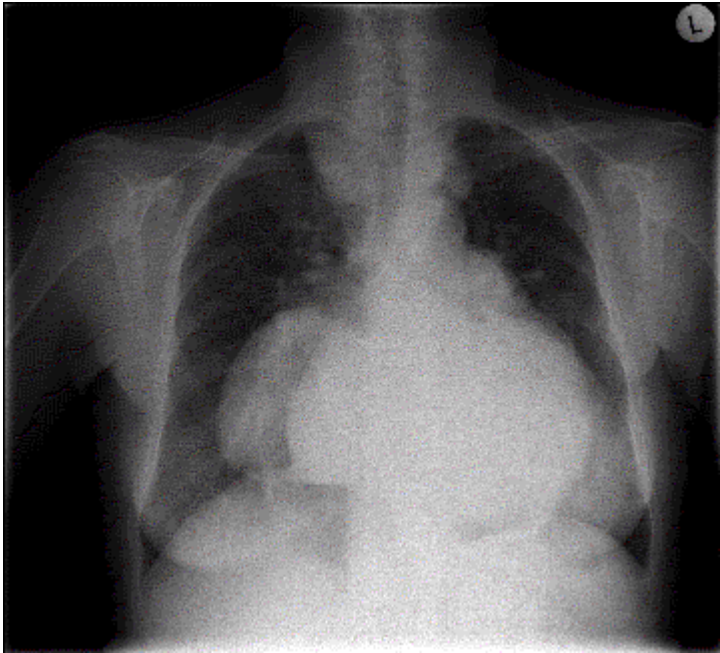
<b>CDA type</b>	<b>I</b>	<b>II</b>	<b>III familial</b>	<b>III sporadic</b>	<b>Variants</b>
<b>Inheritance</b>	Autosomal-recessive	Autosomal-recessive	Autosomal-dominant	Variable	Autosomal-recessive
<b>Cases reported</b>	> 300	~150	3 families	< 20	~70
<b>Morphology</b>	Abnormal chromatin - bridges	Multinuclearity of mature erythroblasts	Giant multi-nucleated erythroblasts	Giant multi-nucleated erythroblasts	CDA I-like CDA II-like others
<b>Gene</b>	<i>CDAN1</i>	Unknown	Unknown	Unknown	Unknown
<b>Locus</b>	15q 15.1.3)	20q (11.2) ?	15q (21-25)	Unknown	Unknown
<b>Associated dysmorphology</b>	Skeleton, Others	Variable, rare	B-Cells Retina	Variable	CNS Others

**Table 2. General definition criteria of the CDAs**

1. Evidence of congenital jaundice/jaundice or a positive family history
2. Evidence of ineffective erythropoiesis
3. Typical morphological appearance of bone marrow erythroblasts, and
4. Exclusion of congenital anemias that fulfill criteria 1 and 2, but were classified according to the underlying defect, such as the thalassemia syndromes, Unclear?) or hereditary sideroblastic anemias



**Fig 2. Large bulks of extramedullary erythropoiesis in a 65 year of female with CDA II.**





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