



## Congenital dyserythropoietic anemia type III

HERBERT SANDSTRÖM,\* ANDERS WAHLIN<sup>o</sup>

\*Family Medicine and <sup>o</sup>Hematology, Medicine, Department of Public Health and Clinical Medicine, Umeå University, Umeå, Sweden

### ABSTRACT

**Background and Objectives.** Congenital dyserythropoietic anemia type III (CDA-III) is a group of very rare disorders characterized by similar bone marrow morphology. The clinical picture is characterized by hemolytic anemia and dramatic bone marrow changes dominated by active erythropoiesis with big multinucleated erythroblasts. The aim of this review is to describe the clinical manifestations, laboratory findings, and management of CDA-III.

**Evidence and Information sources.** The present review critically examines relevant articles and abstracts published in journals covered by the Science Citation Index and Medline. The authors have performed several studies on CDA-III.

**State of Art and Perspectives.** The clinical and laboratory manifestations of CDA-III indicate that the gene responsible for it, which has been mapped to chromosome 15q22, is expressed not only in erythroblasts during mitosis but also in B-cells, and in cells of the retina. Preliminary results indicate genetic and phenotypic similarities between a Swedish and an American family, both with an autosomally dominant inherited form of CDA-III. It is possible that the genetic lesion is identical in these families, but the different phenotypes and modes of inheritance reported among some other cases of CDA-III are probably the results of other genetic lesions. At present, the function of the gene responsible for the Swedish (Västertotten) variant of CDA-III (CDAN3) is unknown and it is an important goal to characterize and clone this gene in order to study its function.

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Dyserythropoiesis is often the result of an acquired defect in a stem cell and its progeny, e.g. myelodysplastic syndrome or leukemia with abnormal development of cell lines, or abnormal environmental conditions within the bone marrow, for instance the toxic effect of chemotherapy or deficiency of vitamin B<sub>12</sub>, folate or iron inhibiting normal cell proliferation. Some of the morphologic changes

in the congenital dyserythropoietic anemias (CDA) resemble those seen in vitamin B<sub>12</sub> or folate deficiency, and disorders of hemoglobin synthesis including thalassemia and hereditary sideroblastic anemia.<sup>1</sup> The term dyserythropoiesis includes abnormalities in both the morphologic and kinetic aspects of erythropoiesis and implies a disturbance during the development of the erythrocyte irrespective of the primary cause. This leads to the production of defective erythroblasts and erythrocytes and an increased rate of phagocytosis of these cells by bone marrow macrophages.<sup>2</sup>

The CDAs are rare inherited disorders with clinical manifestations of ineffective erythropoiesis and a variable degree of anemia. On the basis of the morphologic alterations of the bone marrow and on serologic grounds, in 1968 Heimpel and Wendt classified CDA into three different types.<sup>3</sup> There are, however, cases of CDA that do not meet the full criteria for any of these three types.<sup>4-7</sup>

The clinical severity of these anemias is variable. Some patients are completely asymptomatic and others may require blood transfusions. The diagnosis and classification of the CDAs have so far been based on clinical characteristics, morphologic features of the bone marrow and a few serologic tests. Since clinical and laboratory findings may be subtle, it is reasonable to assume that CDA may be misdiagnosed and confused with other congenital or acquired disorders such as myelodysplastic syndrome, atypical thalassemia, or Gilbert's syndrome.<sup>8</sup> The conspicuous bone marrow findings in CDA-III may also be misdiagnosed as acute erythroleukemia.<sup>9</sup>

In CDA-II the erythrocyte membrane glycoprotein band 3 shows a marked reduction of polylysosamines and migrates slightly faster than normal in SDS polyacrylamide gel electrophoresis. Analysis of N-glycans from CDA-II erythrocyte membranes has revealed incompletely processed N-glycan structures indicating defective glycosylation at the N-acetylglucosaminyltransferase II (GnT-II) and/or  $\alpha$ -mannosidase II (MII) steps,<sup>10</sup> but besides this observation very little is known about the pathologic mechanisms underlying the CDAs.

In 1995, our group located the genetic defect in a Swedish family with CDA-III to chromosome 15q21-25.<sup>11</sup> In an Israeli Bedouin family with CDA-I the genetic lesion was subsequently also located to the long arm of chromosome 15 but in a different region, 15q15.1-15.3.<sup>12</sup> In a study of a large number of Italian families with CDA-II the disease gene has been located to 20q11.2.<sup>13</sup>

Correspondence: Herbert Sandström, MD, Family Medicine, Department of Public Health and Clinical Medicine, Umeå University, 901 85 Umeå, Sweden. Phone: international +46-90-7853521 – Fax: international +46-90-776883 – E-mail: herbert.sandstrom@fammed.umu.se

### **Congenital dyserythropoietic anemia type III**

CDA-III is the rarest form of dyserythropoietic anemia. In a family with 37 affected cases living in the North Swedish county Västerbotten, the disorder is inherited as an autosomal dominant trait with full penetrance of the bone marrow changes.<sup>9,14</sup> The majority of known CDA-III patients belong to the Västerbotten family and two other families, an American family, and an Argentinian family. The mode of inheritance is probably also autosomal dominant in the American family. This family has four affected members, who formed the basis of the original description of CDA-III reported by Wolff and Von Hofe in 1951.<sup>15</sup> The mode of inheritance has not been clarified in eight Argentinian cases but, again, an autosomal dominant mode of inheritance has been suggested.<sup>6,16</sup> Only sporadic cases have been reported from other countries and, overall, fewer than 60 cases are reported in the literature. Some of the sporadic cases of CDA-III seem to have autosomal recessive transmission or be *de novo* spontaneous, dominant mutations.<sup>6, 17-19</sup>

In 1951 Wolff and van Hofe reported the cases of a woman and her three children with mild anemia and remarkable bone marrow findings with abundant multinucleated erythroblasts. The condition was named *familial erythroid multinuclearity* and was later classified as CDA-III. These were the first cases of CDA-III reported in the literature.<sup>15</sup> In 1954 a 35-year old man was investigated at the Department of Medicine in Umeå in northern Sweden because of anemia. The investigations revealed bone marrow changes similar to those occurring in erythroleukemia without there being clinical enlargement of the liver, spleen or lymph nodes. After five years of follow-up there were no signs of progression of the disease. Based on this case Bergström and Jacobsson published a study of the Västerbotten family descending from a couple born in the late 19<sup>th</sup> century. They identified 15 subjects in the family with similar findings and named the disorder *hereditary benign erythroreticulosis*.<sup>9</sup> They showed that the disorder is inherited as an autosomal dominant trait with full penetrance of the bone marrow changes but with minor variations in the clinical picture. We have continued to study this family, which is the largest known CDA-III family, from different perspectives and altogether we have identified 37 subjects with the disease in the family.<sup>11,14,20-22</sup>

### **Clinical findings**

In an investigation of twenty members of the Västerbotten family with CDA-III, 35% regularly suffered weakness, fatigue, or headache. The patients reported more intense symptoms during infections, following trauma, and during pregnancy. Five patients (20%) had received blood transfusions, four of them in late pregnancy or after delivery. Twenty percent reported episodes of abdominal pain interpreted as biliary symptoms and two (10%) had had a cholecystectomy. However, in spite of these symptoms most of the patients regarded themselves as healthy. Six patients (30%) had mild jaundice, but the physical examination was otherwise normal. No patient had splenomegaly.<sup>20</sup>

Although the Swedish and American families are not related to each other the degree of anemia as well as other clinical and laboratory abnormalities in the four patients in the the American CDA-III family discussed above were similar to those found in the Västerbotten family. No information is available concerning urinary hemosiderinuria in the American family, and eye lesions and gammopathy have only been reported in the Västerbotten family. Preliminary results of coupling analyses of a few samples from the American family are compatible with, but do not prove, the occurrence of a common genetic basis of the disorder in the two families (*unpublished data*).

Despite some similarities there are considerable differences in the clinical picture between CDA-III cases reported in the literature. This may indicate a variable phenotypic expression of the same genetic alteration or different genetic abnormalities leading to a similar phenotype. For example, none of the patients described by Wolf and von Hofe nor any of the patients in the Västerbotten family had an enlarged liver or spleen, but splenomegaly was present in eight patients and hepatomegaly in four Argentinian cases.<sup>16</sup> Hepato-splenomegaly was also found in four other cases.<sup>17-19</sup> In the sporadic cases of CDA-III there are some reports of rare clinical findings such as mongoloid facies and mental retardation,<sup>18</sup> malignant T-cell lymphoma,<sup>23</sup> Hodgkin's disease,<sup>24</sup> development of severe iron overload and cirrhosis<sup>25</sup> and extramedullary hematopoiesis.<sup>26</sup>

### **Laboratory findings**

Sixty-five percent of patients belonging to the Västerbotten CDA-III family have mild or moderate anemia. Their median hemoglobin concentration is 118 g/L (range 80-143). The blood film shows moderate or marked anisocytosis and poikilocytosis, basophilic stippling of the red cells and large erythrocytes (macrocytes). The MCV is normal or slightly elevated. The morphology and numbers of leukocytes and platelets are normal. The relative number of reticulocytes is normal or slightly low. Haptoglobin is always low or absent and lactate dehydrogenase elevated. Most patients have a slight increase in bilirubin and aminotransferase levels. There are no significant changes in serum iron, transferrin or ferritin concentrations. Iron staining of the urinary sediment regularly shows hemosiderinuria.<sup>14</sup> Serum thymidine kinase is markedly increased in all patients in the Västerbotten family.<sup>20</sup> The anemia seems to be the result of both ineffective erythropoiesis and hemolysis. The hemolysis is intravascular, as demonstrated by the urinary hemosiderin, explaining why patients with the Västerbotten type of CDA-III do not have any problem with iron overload unlike patients with some other types of CDA.<sup>14</sup>

Agglutination and hemolysis of erythrocytes with anti-i and anti-I antibody was normal in two cases,<sup>22</sup> and increased in two others.<sup>18,25</sup> The acidified serum lysis test (Ham's test) is negative.<sup>14</sup> Studies of the erythrocyte membrane shows only minor changes in membrane proteins. A small difference in relative molecular mass (Mr) has been observed for band 3 in SDS polyacrylamide-electrophoresis in patients with CDA-III.<sup>22</sup> This type of discrepancy has also been observed

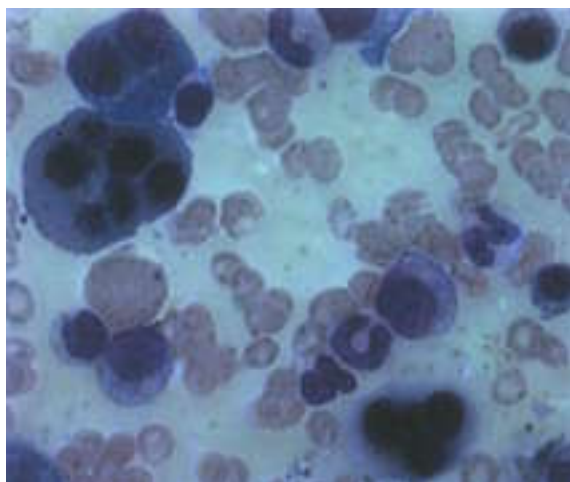


Figure 1. Typical bone marrow smear from a patient with CDA-III and myeloma. To the upper left there is a large multinucleate erythroblast with six nuclei, adjacent to a binucleate plasma cell. May-Grünwald-Giemsa staining (Figure by courtesy of Dr. J. Hultdin).

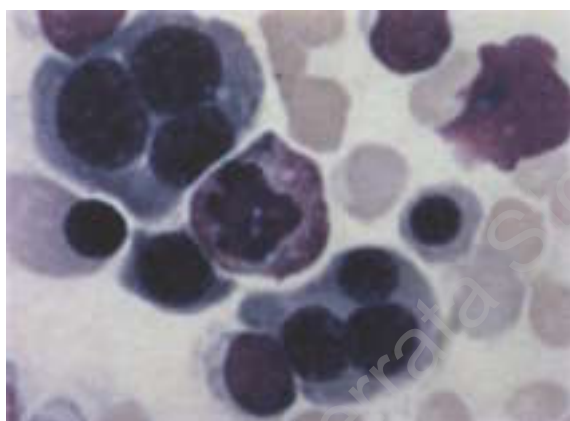


Figure 2. Bone marrow smear from a patient with CDA-III. Two polychromatic multinucleate erythroblasts. May-Grünwald-Giemsa staining. (Figure by courtesy of Dr. J. Hultdin).

in CDA-II, although to a greater extent.<sup>10</sup> The relevance of this finding is unclear but may suggest that glycosylation of band 3 could also be affected in CDA-III.

#### Bone marrow

Light microscopy of bone marrow aspirates shows erythroid hyperplasia with bi- or multinucleated erythroblasts and some giant mononucleated polychromatic erythroblasts.<sup>6</sup> Giant multinucleated erythroblasts, with up to twelve nuclei of variable size, are typical of CDA III (Figure 1 and 2). Anisocytosis, macrocytosis, and poikilocytosis are regularly seen (Figure 3). Myelopoiesis and thrombopoiesis are normal. In the Västerbotten family the bone marrow mor-

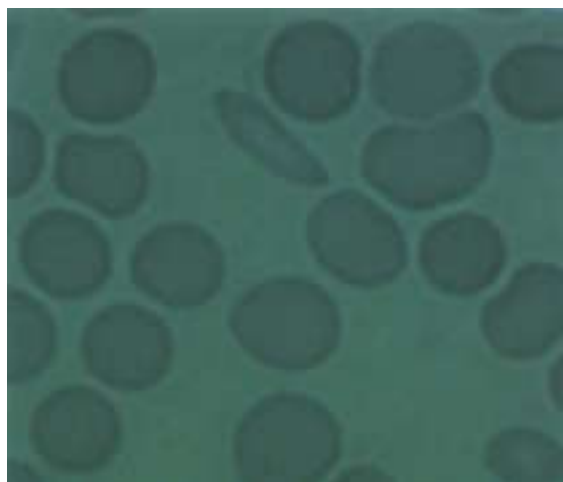


Figure 3. Blood film from a patient with CDA-III showing large erythrocytes, anisocytosis, and poikilocytosis. May-Grünwald-Giemsa staining. (Figure by courtesy of Dr. J. Hultdin).

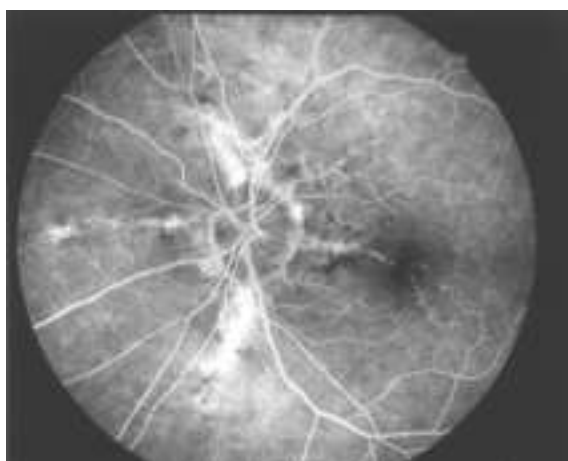
phology was stable over 10-25 years in 17 re-examined patients. Various non-specific dysplastic features can be seen by electron microscopy, e.g. abnormally long intranuclear clefts, abnormally large autophagic vacuoles, iron-laden mitochondria and intracytoplasmic myelin figures. Other findings are duplication or extensive myelinization of large parts of the nuclear membrane and lobulation of the nuclear outline.<sup>22,27</sup> Studies of DNA content of erythroblasts and DNA synthesis show that some of the erythroblasts seem to become arrested during the cell cycle after a period in S-phase. Both mononucleated and multinucleated erythroblasts contain an increased amount of DNA, up to 28c and 48c, respectively, compared to normal (2c). When the separate nuclei within a multinucleated cell are unequal in size, the nuclei usually have different DNA contents and when this difference is marked, the DNA content may differ between <1c and 8c.<sup>18,19,22</sup>

#### Gammopathy and myeloma

Serum electrophoresis was performed in 25 of the Västerbotten patients. In five cases an M-component was present, in all cases of IgG-kappa type. One patient had myeloma, the others had monoclonal gammopathy. In one of the five patients the M-component was also present in the urine. The median age of these patients was 53 years (range 35-80). The patient with myeloma had an M-component for 15 years and smoldering myeloma for 10 years with >30% plasma cells in the bone marrow but without bone disease or any other signs of progress. Another deceased patient with CDA-III in this family had had myeloma with an IgG- $\lambda$  M-component.<sup>14, 21</sup>

#### Eye changes

A few patients belonging to the Västerbotten CDA-III family have developed visual impairment in later life. Ten patients, with and without visual impair-



**Figure 4.** Angioid streaks in a 69-year-old female with CDA-III. Fluorescein angiogram of the left eye. (Photo by courtesy of Dr. O. Sandgren).

ment, were investigated with eye fundus photography and fluorescein angiography. Five healthy relatives were also investigated in the same way. The investigation showed angioid streaks in eight of the investigated patients. (Dr O. Sandgren, personal communication).<sup>21</sup> Skin biopsies taken from five patients to exclude pseudoxanthoma elasticum were negative.

Angioid streaks are a condition usually associated with systemic disorders such as pseudoxanthoma elasticum, Paget's disease of the bone, sickle cell anemia, and  $\beta$ -thalassemia.<sup>28</sup> The characteristic ocular sign is a peripapillary ring with irregularly radiating subretinal linear breaks in Bruch's membrane extending in all directions (Figure 4). The changes are usually bilateral and of varying width from barely visible with an ophthalmoscope to three to four times as wide as the retinal vessels and thus detectable in routine eye fundus investigation. Fluorescein angiography can be used to detect early angioid streaks.<sup>29</sup>

#### **Localization of the disease gene (CDAN3)**

Fifty-six members from the Västerbotten family were studied. Using linkage analyses and recombination data the genetic defect in CDA-III (CDAN3) was located to chromosome 15 (15q21-25) within a distance of 11 cM.<sup>11</sup> Additional investigations have limited the distance to 4.5 cM in 15q22 (Dr. L. Lind, personal communication).

#### **Diagnosis**

Despite the fact that congenital dyserythropoietic anemias are very rare the diagnosis should be considered in neonates, children or adults with anemia and normal to slightly elevated MCV, especially when anisocytosis and poikilocytosis are present and the anemia is familial. Laboratory findings of hemolysis and elevated bilirubin strengthen the suspicion.<sup>8</sup> The investigation should include a blood-count, MCV and red cell volume histogram, reticulocyte count and blood smear. A bone marrow examination is usually diagnostic and should be performed early. A thor-

ough family history focusing on anemia, jaundice and visual impairment is essential. Conditions that may be confused with CDA are MDS, erythroleukemia, congenital and acquired hemolytic disorders, thalassemias, PNH and certain infections such as AIDS, malaria and kala azar.<sup>30</sup> In order to exclude megaloblastic anemia serum B<sub>12</sub> and folate or homocysteine levels should be checked. Hemoglobin electrophoresis should be done in order to demonstrate a possible hemoglobinopathy. Serum thymidine kinase is elevated in CDA-III but has recently been shown to be high also in CDA-I.<sup>31</sup> Measurement of serum thymidine kinase is useful in family investigations when bone marrow aspirate is not available, for instance in small children.<sup>20</sup>

#### **Management**

The anemia in CDA III, if present, is usually mild and does not require any intervention. Transfusion is needed only in exceptional cases with severe anemia, for instance in pregnancy. Iron substitution should not be given if iron deficiency is not present. Increased erythropoiesis may cause increased requirement of folate, and treatment with 5 mg folate per week has been suggested although serum folate is usually normal or only slightly lowered.<sup>30</sup> Our studies show that patients with the Västerbotten type of CDA-III have an increased risk of developing monoclonal gammopathy, myeloma and eye changes (angioid streaks).<sup>21</sup> Patients with M-proteins should be examined annually with routine laboratory investigations including electrophoresis. Eye fundus examination by an ophthalmologist is recommended at the time of diagnosis. Thereafter, fundus photography is recommended every second to third year in patients over 50 years of age. Photocoagulation of early neovascular changes in the retina seems to prevent visual impairment in patients with angioid streaks associated with other disorders.<sup>32</sup> Although the benefit of such procedures has not been proven in angioid streaks associated with CDA-III they may be useful also in this condition. Current data do not indicate any benefit of regular follow-up in younger patients without gammopathy and eye lesions.

#### **Perspectives**

Although the genetic lesion causing CDA-III has been mapped to chromosome 15q22 in the Västerbotten family the function of the gene is completely unknown. The occurrence of multinucleated erythroblasts containing nuclei of different degrees of maturation may indicate that the gene product causes a disturbance during mitosis in erythropoietic cells. However, the gene operating in CDA-III obviously also causes abnormal functions in other cell lines, as demonstrated by the occurrence of monoclonal gammopathy and angioid streaks among the same members of the family who carry the CDA-III trait. The American CDA-III family, originally described by Wolff and von Hofe, seems to have the same phenotype as the Västerbotten family, although monoclonal gammopathy and angioid streaks have not been reported in this family. It is possible that the genetic lesion in the American and Västerbotten families is identical, but the different phenotypes report-

ed among some other cases of CDA-III are probably the result of other genetic lesions. Cloning of the gene is essential in order to study the function of the gene, which may give valuable information about mechanisms involved in mitosis.

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*The two authors both made significant contributions in all aspects.*

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