Congenital dyserythropoietic anemia type II: epidemiology, clinical appearance, and prognosis based on long-term observation

Hermann Heimpel, Volker Anselstetter, Ladislav Chrobak, Jonas Denecke, Beate Einsiedler, Kerstin Gallmeier, Antje Griesshammer, Thorsten Marquardt, Gritta Janka-Schaub, Martina Kron, and Elisabeth Kohne

Congenital dyserythropoietic anemia type II (CDA II) is the most frequent type of congenital dyserythropoietic anemia. More than 200 cases have been described, but with the exception of a report by the International CDA II Registry, these reports include only small numbers of cases and no data on the lifetime evolution of the disease. Since 1967, we were able to follow 48 cases of CDA II from 43 families for up to 35 years. All patients exhibit chronic anemia of variable severity requiring regular red cell transfusions only in a minority of children; 60% developed gallstones before the age of 30 years, and 16 patients had cholecystectomy between 8 and 34 years of age. Iron overload was a frequent complication. In 16 cases, iron depletion started between 7 and 36 years. Three patients died from secondary hemochromatosis. Splenectomy, performed in 22 cases, led to moderate increases in hemoglobin values and eliminated the need for transfusions but did not prevent further iron loading. The current recommendation is to consider splenectomy if the anemia compromises patients' performance, and to manage iron overload according to the guidelines derived from patients with thalassemia. (Blood. 2003;102:4576-4581)

Introduction

The congenital dyserythropoietic anemias (CDAs) comprise a group of rare hereditary disorders of erythropoiesis, characterized by ineffective erythropoiesis as the predominant mechanism of anemia and by distinct morphologic abnormalities of the majority of erythroblasts in the bone marrow. The term was first used by Crookston et al[1] (for cases later classified as CDA II) and by Wendt and Heimpel[2] (for cases later classified as CDA I), but a few reports of similar cases had been published previously.[3] In 1968, we proposed classifying these disorders into 3 types.[3] Although initially proposed as a working classification, it was widely accepted and is still used today in clinical practice.[4,5]

CDA II, also known as hereditary erythroblastic multinuclearity with a positive acidified-serum test (HEMPAS),[6] is the most frequently encountered disorder of the CDA group.[4,5,7,8] The leading morphologic abnormality is binuclearity or multinuclearity occurring in 10% to 50% of mature erythroblasts, with equal DNA content in both nuclei.[9] Electron microscopy (EM) shows a double membrane close to the cell membrane of mature erythroid cells,[10] which is due to residual endoplasmic reticulum.[11] Band 3 appears thinner and shows faster migration on sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE).[12] Red cells of patients with CDA II retain throughout life a very high agglutinability by anti-i sera.[6]

The abnormalities of the CDA II red blood cell membrane are due to abnormal processing of N-glycans.[13] Band 3 and band 4.5 glycoproteins carry truncated polylactosamine structures, while glycolipids are sometimes overglycosylated.[14] An association to a gene locus on chromosome 20 (q11.2) was described in families from Southern Italy.[7,15]

After our description of the first families in 1968,[3,16] we observed many more cases, mostly in residents of Germany, but also from Austria, Switzerland, and the Czech Republic, and we were able to follow some patients up to 35 years. Data from these patients as well as from case reports in the literature were collected in the German CDA registry, set up in 1993. Here we report epidemiologic data, clinical manifestations, and diagnostic features of 48 patients with CDA II. Particular emphasis is given to the course of the disease as a basis for management of these patients.

Patients, materials, and methods

Diagnosis of CDA II was based on the criteria shown in Table 1. Data were extracted from files of the institutions and physicians responsible for patients' management from 1950 to 2002, in addition to the personal observations of one of the authors (H.H.) from the university hospitals of Freiburg and Ulm, Germany. Informed consent was obtained for additional blood samples taken for research objectives. A code identifying the family, the cases, and their relatives was assigned to each individual. Family trees were constructed by Cyrillic 2.1.2 (Cherwell Scientific Publishing, Oxford, United Kingdom). Data from 14 patients from 11 families had been published before.[3,16-25]

Data used to analyze the course of disease were retrieved both by retrospective search for patients' charts and other sources and by prospective monitoring after the diagnosis had been made and the patient was reported to the registry. Observation times, defined as the interval between the first and the last set of laboratory tests, ranged from 1 to 48 years (median, 17 years). For presentation of relevant parameters, all available data after the age of 3 months and before splenectomy were used. Data

From the Abteilung Innere Medizin III, the Kinderklinik, and the Abteilung Biometrie, Universität Ulm, Ulm, Germany; Department of Hematology, Charles University Faculty of Medicine, Hradec Králové, Czech Republic; Klinik für Kinderheilkunde, Universität Münster, Münster, Germany; and Universitäts-Kinderklinik, Hamburg, Germany.

Submitted February 25, 2003; accepted August 6, 2003. Prepublished online as *Blood* First Edition Paper, August 21, 2003; DOI 10.1182/blood-2003-02-0613.

Reprints: Hermann Heimpel, Abteilung Innere Medizin III (Hematology/Oncology) der Universität Ulm, Robert Koch Str 8, D-89081 Ulm, Germany; e-mail: hermann.heimpel@medizin.uni-ulm.de.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 U.S.C. section 1734.

Table 1. Diagnostic criteria for CDA II: presence of all A-criteria and of at least one B criterion is required

A criteria
A1: Evidence of congenital anemia/jaundice or heredity
A2: Evidence of ineffective erythropoiesis
A3: Typical morphologic appearance of bone marrow erythroblasts, with at least 10% binucleated red cell precursors
B criteria
B1: Positive acid lysis with at least 20% of normal sera
B2: Typical abnormality of bands 3 and 4.5 in SDS-PAGE
B3: Double membrane running internally from the cell membrane of late erythroblasts seen by electron microscopy (EM)

measured after transfusion or at the time of aplastic crisis or severe independent illness, such as neoplasia, were excluded.

General diagnostic procedures were dependent on the time period in which they were applied. Serum haptoglobin was measured by radial immunodiffusion or nephelometry; concentrations below the threshold of determination of the individual laboratory (mostly 0.2 g/L) were defined as absent. Estimation of red cell survival followed standard procedures.[26]

Acidified-serum tests and agglutination by anti-i were performed by means of the technique of Crookston et al[27] or a modification previously described.[28] Titers of agglutination by anti-i were regarded as indicative of CDA II if patients' red cells were agglutinated by at least an 8-fold dilution of the serum as compared with healthy adult controls. SDS-PAGE and preparations of bone marrow specimens for EM were done as previously described.[12,29]

MS–Access 2000 (Microsoft, Seattle, WA) was used as the data bank management system, and statistical analyses were performed by means of SAS version 8.2 (SAS Institute, Cary, NC). Event rates were calculated according to the method of Kaplan-Meier, and subgroups were compared by means of the log-rank test. If not otherwise stated, the Spearman rank correlation coefficient was used to correlate laboratory values and the Wilcoxon signed rank test was used to compare paired data.

Results

Epidemiology and inheritance

Detailed information from patients seen in Ulm and/or obtained by correspondence is available for 48 cases from 43 families. Families 37, 5, and 1 were resident in Germany, Austria, and the Czech Republic, respectively. The male-female ratio was 1:0.74. Ethnic origin other than those of the countries of residence included the following (with number of patients in parentheses): Albanian (1), Greek (2), Italian (2), Romanian (1), Sinti (1), Spanish (1), and Turkish (2). In 14 German or Austrian families, ancestry could be traced back to at least 3 (maximum, 11) generations without showing ethnicity from non-German linguistic regions. Consanguinity of parents of the probands was detected in 2 families. Blood counts and serology data are available from 37 and 29 first-degree relatives, respectively. As expected from the autosomal recessive heredity of CDA II, there was no unexplained chronic anemia or hyperbilirubinemia, except in affected siblings of probands. Agglutination by anti-i was not increased, and acid-serum tests were negative. In 3 families, stillbirths were reported in siblings of the patients. Ten female and 3 male patients had 13 and 5 children, respectively.

Key observations at diagnosis

Anemia and/or jaundice were usually recognized in childhood or in young adults. Age at first diagnosis of CDA II ranged from 0.1 to 78 years (median, 18.2 years) (Figure 1A). In 2 exceptional cases, long-standing moderate anemia was investigated in the workup of an unrelated disorder, and the correct diagnosis was recognized at 62 and 78 years, respectively. Previous incorrect diagnoses included hemolytic anemia, thalassemia, hereditary spherocytosis, iron deficiency, and hepatitis. Some patients had been treated with iron, a variety of vitamins, or prednisone. Four had additional congenital malformations such as a ventricular septal defect, hemihypertrophy, or mental retardation.

Pertinent laboratory data derived from means in individual cases are shown in Table 2. Hemoglobin concentration was somewhat lower in children than in later life ($P = .054$; Wilcoxon signed rank test), but as shown by the distribution of relative frequencies (Figure 1B), this does not account for the variable severity of the anemia. With one exception, hemoglobin concentrations were below the age-specific reference intervals in children and adolescents. Relative reticulocyte counts were normal or moderately increased; only one case showed 5% to 10% at repeated determinations (Figure 1C). There were always distinct anisocytosis and poikilocytosis without specific types of poikilocytes, with basophilic stippling of cells and few, occasionally binucleated, mature erythroblasts. Mean cellular volume (MCV) and mean cellular hemoglobin (MCH) were normal in 42 patients, but were between 75 and 80 fL or 25 and 29 pg in 5 children or adolescents, and more than 100 fL and 35 pg in 2 adult cases, respectively. White blood cell (WBC) and platelet counts were in the normal range throughout. Red cell survival was moderately shortened in 13 of 14 cases, with apparent half-times between 14 and 28 days (normal, 25 to 35 days).

Total serum bilirubin at the time of diagnosis was moderately increased in 90% of all cases. This was due exclusively to an increase of the indirect reacting fraction. When follow-up data were included, all cases showed hyperbilirubinemia. Independently of the fluctuations observed, some cases had consistently higher values than others. Haptoglobin was low or absent in 47 of 48 patients.

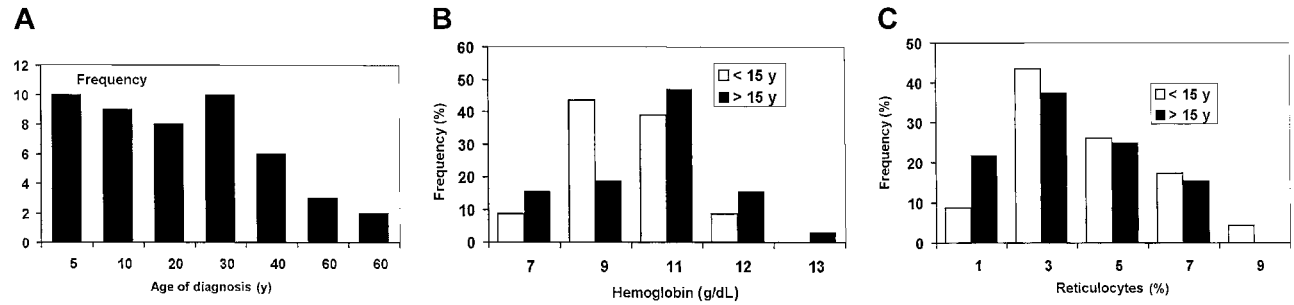

Figure 1. Age at diagnosis, hemoglobin concentration, and reticulocyte counts. (A) Age when the diagnosis of CDA II was made. (B) Hemoglobin concentration. (C) Relative reticulocyte counts.

Table 2. Pertinent laboratory values

	Age younger than 15 years				Age older than 15 years			
	No.	Median	Minimum	Maximum	No.	Median	Minimum	Maximum
Hemoglobin concentration, g/dL	23	9.1	6.8	12.0	32	9.8	6.1	12.7
Erythrocyte count, $\times 10^{12}$/L	22	3.1	2.4	4.4	32	3.2	1.9	4.4
Mean cellular volume, fL	20	89	76	100	30	91	77	110
Mean cellular hemoglobin, pg	21	29	25	34	30	31	25	34
Reticulocyte count, %	21	3.0	1.1	8.6	32	2.7	0.5	6.5
Total bilirubin, mg/dL	22	2.0	0.7	5.1	31	3.0	0.7	8.5

In 6 cases, data before splenectomy were not available.

Specific abnormalities of CDA II

All 48 patients had typical bone marrow findings and laboratory evidence of ineffective erythropoiesis. Bone marrow specimens showed distinct hypercellularity due to erythroid hyperplasia. Ratio of erythropoietic to granulopoietic cells (E/G ratio) and varied between 3 and 10, compared with a normal range of between 0.2 and 1. Characteristic abnormalities of mature erythroblasts with 10% to 45% (median, 20%) multinucleated cells were observed as previously described.[3,9] Pseudo-Gaucher macrophages with birefringent material were seen in most cases.

Not all parameters designated B criteria in Table 1 were identified in each patient. B2 (SDS-PAGE, 23 of 23) and B3 (EM, 11 of 11) criteria were always positive. Two of 47 patients with a negative acidified-serum test (B1) could not be retested with a panel of sera known to react with other cases of CDA II. Both showed a typical pattern in SDS-PAGE. Controls including red cells of patients with CDA I or CDA variants were negative. In 45 of 45 cases, red cells were agglutinated by anti-i titers comparable to normal cord blood erythrocytes. Any B criterion remained positive on subsequent controls, except for one patient in whom the acid-serum test became negative when he developed warm antibody autoimmune hemolytic anemia.

Prognosis and course of disease

At present, 10 patients are older than 50 years, with 4 patients being older than 70 years. Most adolescents had normal school education, but expressed moderate fatigue, and reported that their physical fitness was diminished compared with their classmates. Three male patients died between the age of 25 and 32 years of liver cirrhosis and portal hypertension caused by secondary hemochromatosis, and 4 other patients died of unrelated causes between the age of 40 and 77 years. Two patients had malignant lymphoma detected at age 42 and 68 years, respectively. No other clonal diseases of the lymphohematopoietic system were observed.

Moderate splenomegaly was present before or at diagnosis in 26 patients. In the majority of the other patients, splenomegaly became apparent in the 3 first decades of life. Gallstones were found in 22 of 39 patients before the age of 40 years and were sometimes detected in childhood or adolescence (Figure 2A). Cholecystectomy was performed in 16 patients between the ages of 8 and 34 years (median, 25.7 years).

Most patients developed iron overload. Probability of ferritin values of at least 300 or at least 1000 μg/mL (at least 300 or 1000 ng/mL) were dependent on age of patient, as shown in Figure 2B. Only data obtained before splenectomy and before a first course of iron depletion were used. The increment of ferritin usually started at the age of 20 years. However, there were great interindividual differences, with 3 of 14 evaluable patients aged 40 years or older at the last determination not reaching values above 300 μg/mL (above 300 ng/mL).

Growth was normal in all but one of the cases for which we had information after the age of 20 years. Organ damage due to secondary hemochromatosis was rather infrequent, possibly owing to the policy of starting iron depletion if ferritin values approached 1000 μg/mL (1000 ng/mL) (Table 3). However, a small fraction of patients showed consequences of iron overload in early adulthood. Rare complications included severe aplastic crisis after parvovirus B 19 infection in 4 patients and paravertebral extramedullary hematopoiesis in 2 patients.

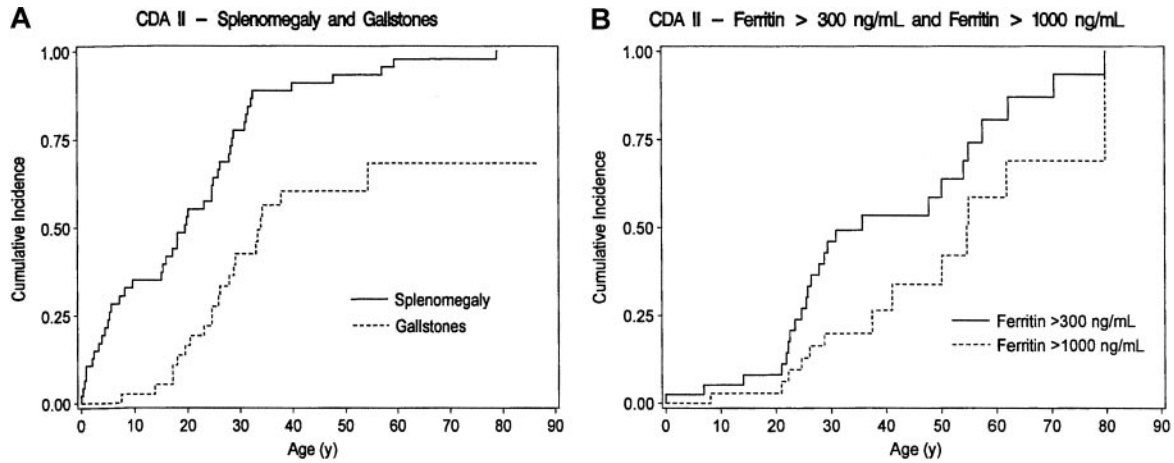

Figure 2. Splenomegaly/gallstone detection; risk of iron overload. (A) Detection of splenomegaly and of gallstones. (B) Risk of iron overload dependent on age. This shows that the probability of reaching concentrations of 300 μg/mL and 1000 μg/mL (300 ng/mL and 1000 ng/mL) serum ferritin is dependent on age.

Table 3. Functional consequences of anemia or secondary hemochromatosis

Disorder	No. patients with disorder/total no. patients	Age of first presentation, years
Growth retardation*	1/41	NA
Liver cirrhosis	9/43	16-65
Heart failure	4/45	22-52
Diabetes	9/44	16-70
Hypothyroidism	2/43	28-49
Hypogonadism	3/40	20-49

NA indicates not applicable.
*Only cases with patients older than 20 years of age at the time of analysis.

Therapy

In 7 patients, repeated red cell transfusions were given up to the age of 7 years, but were subsequently needed only in aplastic crisis, in severe infections, during pregnancy, or at major surgery, for example, when the gallbladder and/or spleen were removed. Two of 21 children for whom we had sufficient data before splenectomy were transfusion dependent.

Splenectomy was performed in 22 patients at the age of 1 to 50 years (median, 19.9 years). In 7 cases, the spleen had been removed before the diagnosis of CDA II was made, on the basis of a tentative diagnosis of hereditary spherocytosis or an unclassified hemolytic anemia. Hemoglobin concentration improved in all patients, on average from 91.70 to 103.4 g/L (9.17 to 10.34 g/dL) ($P < .001$). In all but one case, however, hemoglobin levels remained below sex-matched reference values. Increased hemoglobin concentration was sustained during observation up to 25 years. Platelet counts increased to maximal values of 1200×10^9/L, and in some cases platelet counts remained elevated for many years. Reticulocyte counts decreased from 0.0332 to 0.0145 (3.32% to 1.45%) ($P = .008$), and bilirubin fell from 39.0 to 22.9 μM (2.28 to 1.34 mg/dL) ($P = .002$). Splenectomy did not prevent further loading of iron. Thirteen of 17 patients with ferritin values available after splenectomy showed elevated values. Eight received treatment with deferoxamine. Details of the effect of splenectomy as well as of iron depletion in both type II and type I CDA will be reported in detail in another publication.

Thirteen patients were treated with deferoxamine in doses recommended for treatment of secondary hemochromatosis in thalassemia major.[30] Treatment was usually begun when plasma ferritin reached a serum concentration of 1000 μg/L (1000 ng/mL). Age at initiation of deferoxamine therapy ranged from 7 to 35 years (mean, 20 years; median, 21 years). Reduction of elevated serum ferritin concentrations was achieved in all patients, while normal ferritin concentrations (below 300 μg/L [below 300 ng/mL]) were reached in all patients with satisfactory compliance. Three splenectomized patients underwent regular phlebotomies of 200 to 300 mL every 4 to 6 weeks, with normalization of ferritin and transferrin iron saturation.

Discussion

The classification of the congenital dyserythropoietic anemias was originally based on distinct differences in the morphology of the bone marrow erythroblasts.[3] CDA II, the most frequent type, is the only entity characterized by additional diagnostic features of high specificity. In our patients, acidified-serum tests ("Ham tests") were unequivocally positive, except in 2 who could not be retested with more sera. Red cells of all our patients with CDA II, but of no patient with CDA I, analyzed by SDS-PAGE showed the typical pattern as previously described. Both tests, as well as additional changes such as absent lectin binding,[25] reflect the same membrane abnormality and are the result of the common, although variable, underglycolysation of band 3 glycoproteins.[7,14,31]

In addition to our original classification, CDA type IV has been described with typical morphologic features of CDA II but a negative acidified-serum test.[32] Some of these reports were later retracted when retesting with more sera gave positive results.[33] However, we and others[34,35] have observed families fulfilling all A criteria as stated in Table 1 but whose acid-serum tests are consistently negative. At present, we follow such patients from 3 families. These patients display a severe clinical course and require regular transfusions. In none of them has any other B criterion been positive. The definition of CDA II should be therefore used as a synonym of HEMPAS, and the suspected diagnosis based on A criteria must be confirmed by at least one B criterion, preferably SDS-PAGE and/or the acidified-serum test with the use of appropriate positive and negative controls. Since agglutination scores by anti-i were invariably extremely high, a normal score excludes the diagnosis. Our data are essentially in agreement with the observations of the International CDA II Registry, although their compilation included cases based on morphology only. Western blots of red cells detecting proteins of the endoplasmatic reticulum, not done in our study, may become an additional specific criterion.[36]

While CDA II is known as a "rare disease," prevalence data are not available. We identified 205 cases from 160 families published as case reports, excluding repeated publications of the same individuals but including the cases analyzed in this paper. In addition, the majority of the 98 cases compiled by the International CDA II Registry have not been published as case reports, and no cases described here were included in its recent publication[36] (A. Iolascon, personal written communication, 2003). Gene frequency seems to be higher in Southern Italy than in other regions of Europe.[4,37] The present report is the first to show that CDA II is not so rare in central Europe as formerly thought, with negative evidence in many families that their ancestry may derive from Mediterranean countries. Two families from Italy[38] and one of our German families (A. Iolascon, personal written communication, 1998) failed to show an association to the gene locus at 20(q11.2). We did not see a conspicuously high frequency of CDA II in the large Turkish, Italian, and Greek populations living in Austria and Germany, but the ascertainment rates may be different and the number of observations is too small to draw positive conclusions.

The observations reported here allow some conclusions for the diagnosis and management of patients with CDA II. In many cases, the diagnosis was first recognized in adults, although anemia and/or hyperbilirubinemia had been known for many years. Similar delay has been reported previously.[36,39] In some patients, erroneous diagnoses such as hereditary spherocytosis were made many years after CDA II had been recognized as a novel entity. The diagnosis should be suspected in any case of congenital anemia with indirect hyperbilirubinemia, inadequate reticulocytosis, and low or absent plasma haptoglobin. Red cell morphology in the smear is always abnormal, but of low specificity, except if a typical binucleated normoblast is found. Distinct microcytosis/hypochromia with MCV below 70 fL or MCH concentration below 25 pg is not present in CDA II, except in rare cases with additional iron deficiency or heterozygous thalassemia.[40] The severity of the anemia is variable, with about 10% of patients in both Iolascon's and our series requiring regular red cell substitution in infancy and

childhood, while others have only moderately decreased hemoglobin values throughout their life (Figure 1B). The parameters underlying this heterogeneity are not understood. Although the comparison with the data from the International CDA II Registry (the majority of whose cases have been reported from Southern Italy) is limited by the fact that the registry reported mean and standard deviations without showing age-adjusted distributions, the magnitude of the abnormalities in hemoglobin concentrations, red cell indices, and reticulocyte counts is not different. Total bilirubin concentrations seem to be higher in the registry cases, possibly by differences in expression of uridine diphosphate glucuronosyl transferase (UGT1A), as shown by others.[41] No phenotypic differences were noted between patients of German or Austrian ancestry compared with those of Mediterranean ethnicities. Since the information we have on linkage to the *CDAN2* gene on 20q is derived only from single cases, phenotypic and potential genotypic heterogeneity cannot be correlated in our patients.[42] The hypothesis that 20q unlinked cases have a more severe disease[36] is still a subject of debate, and since this linkage probably reflects a founder effect,[37] more non-Italian families have to be analyzed to test this hypothesis.

Splenomegaly, cholelithiasis, and iron overload are all secondary consequences of ineffective erythropoiesis and increased peripheral red cell destruction. Splenomegaly was documented on follow-up in almost all patients (Figure 2A). Thus, absence of splenomegaly in adolescents and adults should always raise doubt about the diagnosis. As shown in Figure 2A, gallstones are usually detected in the first 40 years of life and not infrequently in children. Interestingly, cholelithiasis was much less frequent in CDA I, having been found in 3 of 20 in our own patients followed for up to 35 years (data not shown), as well as in 3 of 16 patients of a Bedouin tribe.[43]

The main problem encountered by patients after the first years of life is iron loading, which is also seen in patients without ongoing need for transfusion. The fact that patients with both CDA I and CDA II load iron in a manner similar to those with other chronic states of ineffective erythropoiesis has been known since the early observations.[3,6,16] As seen from Figure 2B, iron accumulates steadily throughout life, with kinetics similar to untreated hereditary hemochromatosis. There is distinct variability among individuals, which seems not to be explained by *HFE* gene polymorphism.[36,43,44] Three young male patients who died from liver cirrhosis and heart failure were diagnosed between 1971 and 1980, and although high values of ferritin and transferrin saturation were known, they did not undergo iron depletion. Analyzed retrospectively, organ dysfunction as shown in Table 3 occurred in patients with poor control of ferritin values. Adequate treatment with deferoxamine or regular phlebotomies in compliant patients achieved normal ferritin concentrations. These results confirm evidence from case reports that iron depletion postulated by Cazzola et al[45] is effective in reversing iron overload in patients with CDA.[24,46]

In agreement with the data of the International CDA II Registry on 26 patients and the results of 41 patients published as case reports, a moderate and sustained increase in hemoglobin concentration after splenectomy was seen in all 22 patients. In 3 of them, red cell survival was moderately shortened before splenectomy and normal afterwards.[47] Similar data have been previously reported,[48] demonstrating that, as in hereditary spherocytosis, abnormal CDA II erythrocytes may survive normally in an asplenic individual. No overwhelming bacterial infections were observed. Portal vein thrombosis occurred in 2 patients after splenectomy and also in some cases of CDA I.[49] In contrast to a CDA II–like variant with negative B criteria,[50-52] prominent peripheral postsplenectomy erythroblastosis was absent. Contrary to our expectations, splenectomy did not prevent further iron loading, even in those cases where hemoglobin concentrations became nearly normal. 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.

Confirmation of CDA II should be achieved in the first year of life for counseling of the parents and planning of lifelong therapy and follow-up. To prevent organ damage, lifelong control of iron stores is required, with regular check of ferritin values. Iron depletion has to be started at the latest when ferritin approaches a level of 1000 μg/mL (1000 ng/mL). Since data correlating serum ferritin values to tissue iron are not available in CDA, prospective studies using noninvasive techniques of liver iron determination[53] are required to formulate more exact recommendations. The main utility of splenectomy is abrogation of transfusion requirements in more severe cases and increase of the hemoglobin concentration to render regular phlebotomy possible. In other patients, we followed the guidelines for mild cases of hereditary spherocytosis,[54] but follow-up data from more patients will be needed to give evidence-based recommendations for the total management of patients with CDA II.

Acknowledgments

We express our gratitude for excellent technical work to Helga Dietrich (serology), Christine Eggl (data bank programming), and Rosi Leichtle (data management). Data and blood samples of patients were obtained from many physicians taking care of the patients. We thank all our colleagues for their cooperation. This work could not have been achieved without their interest and support.

References

1. Crookston JH, Godwin TF, Wightmann KJR, Dacie JV, Lewis SM, Patterson M. Congenital dyserythropoietic anemia. Abstr XIth Congress Int Soc Hematol, Sydney, Australia. 1966.
2. Wendt F, Heimpel H. Kongenitale dyserythropoetische Anämie bei einem eineiigen Zwillingspaar. Med Klinik. 1967;62:172-177.
3. Heimpel H, Wendt F. Congenital dyserythropoietic anemia with karyorrhexis and multinuclearity of erythroblasts. Helv Med Acta. 1968;34:103-115.
4. Wickramasinghe SN. Congenital dyserythropoietic anaemias: clinical features, haematological morphology and new biochemical data. Blood Rev. 1998;12:178-200.
5. Delaunay J, Iolascon A. The congenital dyserythropoietic anaemias. Baillieres Best Pract Res Clin Haematol. 1999;12:691-705.
6. Crookston JH, Crookston MC, Burnie KL, et al. Hereditary erythroblastic multinuclearity associated with a positive acidified-serum test: a type of congenital dyserythropoietic anemia. Br J Haematol. 1969;17:11-26.
7. Iolascon A, D'Agostaro G, Perrotta S, Izzo P, Tavano R, Miraglia del Giudice B. Congenital dyserythropoietic anemia type II: molecular basis and clinical aspects. Haematologica. 1996;81:543-559.
8. Heimpel H, Maier K. Congenital dyserythropoietic anemia: a first approach to epidemiology [abstract]. Haematologica. 1999;84(EHA-abstracts):194.
9. Queisser W, Spiertz E, Jost E, Heimpel H. Proliferation disturbances of erythroblasts in congenital dyserythropoietic anemia type I and II. Acta Haematol. 1971;45:65-76.

10. Heimpel H, Forteza-Vila J, Queisser W. Morphological aberrations of the erythroblasts in congenital dyserythropoietic anemia type I and II [abstract]. Abstr Vol XIII Int Congr Int Soc Hematol. Munich 1970. 1970;371.
11. Alloisio N, Texier P, Denoroy L, et al. The cisternae decorating the red blood cell membrane in congenital dyserythropoietic anemia (type II) originate from the endoplasmic reticulum. Blood. 1996;87:4433-4439.
12. Anselstetter V, Horstmann K, Heimpel H. Congenital dyserythropoetic anaemia, types I and II: aberrant pattern of erythrocyte membrane proteins in CDA II, as revealed by two-dimensional polyacrylamide gel electrophoresis. Br J Haematol. 1977;35:209-215.
13. Fukuda MN, Papayannopoulou T, Gordon-Smith EC, Rochant H, Testa U. Defect in glycosylation of erythrocyte membrane proteins in congenital dyserythropoietic anaemia type II (HEMPAS). Br J Haematol. 1984;56:55-68.
14. Zdebska E, Anselstetter V, Pacuszka T, et al. Glycolipids and glycopeptides of red cell membranes in congenital dyserthropoetic anemia type II (CDA II). Br J Haematol. 1987;66:385-391.
15. Iolascon A, Miraglia del Giudice E, Perrotta S, Granatiero M, Zelante L, Gasparini P. Exclusion of three candidate genes as determinants of congenital dyserythropoietic anemia type II (CDA-II). Blood. 1997;90:4197-4200.
16. Heimpel H, Wendt F, Klemm D, Schubothe H, Heilmeyer L. Kongenitale dyserythropoietische Anämie. Dtsch Arch Klin Med. 1968;215:174-194.
17. Minssen M, Klaus D. Familiäre Vielkernigkeit der Erythroblasten mit makrozytärer Anämie durch ineffektive Erythropoese und gesteigerte Hämolyse. Med Welt. 1968;19:2280-2283.
18. Hartwich G, Wolf F, Becker J, Keilhack W, Schwanitz G. Kongenitale dyserythropoietische Anämie. Blut. 1971;23:347-358.
19. Schatanek W, Hufnagl HD, Meiser RJ, Berberich R. Kongenitale dyserythropoetische Anämie mit Eisenspeicherkrankheit. Muench Med Wochenschr. 1972;114:1933-1936.
20. Kurz R, Huber C, Reinken L, Huber H. Kongenitale dyserythropoetische Anämie II: Pädiatrie und Pädologie. 1973;8:135-146.
21. Hartwich G, Riemann J, Kronert E. Kongenitale dyserythropoietische Anemia Typ II: ein weiterer Fallbericht. Med Klinik. 1976;71:2117-2122.
22. Chrobak L, Radochova D, Smetana K. Congenital dyserythropoietic anaemia type II (HEMPAS) in three siblings. Folia Haematol (Leipz). 1980; 107:628-640.
23. Koehler M, Schmidt-Riese L, Brandeis WE. Morphological studies of erythrocytes in congenital dyserythropoietic anemia type II [in Polish]. Acta Haematol Pol. 1990;21:144-152.
24. Hofmann WK, Kaltwasser JP, Hoelzer D, Nielsen P, Gabbe EE. Successful treatment of iron overload by phlebotomies in a patient with severe congenital dyserythropoietic anemia type II [letter]. Blood. 1997;89:3068-3069.
25. Denecke J, Kranz C, Conradt H, Heimpel H, Weglage J, Marquardt T. Congenital dyserythropoietic anemia typ II (CDAII/Hempas), new biochemical insight in a disorder or glycosylation [abstract]. J Inherit Metabol Dis. 2001;111.
26. International Committee for Standardisation in Hematology. Recommended methods for radioisotope red-cell survival studies. Br J Haematol. 1980;45:659-666.
27. Crookston JH, Crookston MC, Rosse WF. Red-cell abnormalities in HEMPAS (hereditary erythroblastic mulitnuclearity with a positive acidified-serum test). Br J Haematol. 1972;23(suppl): 83-91.
28. Erdmann H, Heimpel H, Buchta H. Positiver Säureserumtest und erhöhte Agglutinabilität durch Anti-i bei Patienten mit kongenitaler dyserythropoietischer Anämie. Klin Wochenschr. 1970;48: 569-570.
29. Heimpel H, Forteza-Vila J, Queisser W, Spiertz E. Electron and light microscopic study of the erythropoiesis of patients with congenital dyserythropoietic anemia. Blood. 1971;37:299-310.
30. Wonke B. Clinical management of beta-thalassemia major. Semin Hematol. 2001;38:350-359.
31. Fukuda MN. HEMPAS disease: genetic defect of glycosylation. Glycobiology. 1990;1:9-15.
32. McBride JA, Wilson WE, Baillie N. Congenital dyserythropoietic anaemia: type IV [abstract]. Blood. 1971;38:837. Abstract 153.
33. Seip M, Skrede S, Bjerve K, et al. A case of variant congenital dyserythropoietic anemia revisited. Scand J Haematol. 1982;28:278-280.
34. Benjamin JT, Rosse WF, Dalldorf FG, McMillan CW. Congenital dyserythropoetic anemia: type IV. J Pediatr. 1975;87:210-216.
35. Eldor A, Matzner Y, Kahane I, Levene C, Polliack A. Aberrant congenital dyserythropoietic anemia with negative acidified serum tests and features of thalassemia in a Kurdish family. Isr J Med Sci. 1978;14:1138-1143.
36. Iolascon A, Delaunay J, Wickramasinghe SN, Perrotta S, Gigante M, Camaschella C. Natural history of congenital dyserythropoietic anemia type II. Blood. 2001;98:1258-1260.
37. Iolascon A, Servedio V, Carbone R, et al. Geographic distribution of CDA-II: did a founder effect operate in Southern Italy? Haematologica. 2000; 85:470-474.
38. Iolascon A, De Mattia D, Perrotta S, et al. Genetic heterogeneity of congenital dyserythropoietic anemia type II [letter]. Blood. 1998;92:2593-2594.
39. Greiner TC, Burns CP, Dick FR, Henry KM, Mahmood I. Congenital dyserythropoietic anemia type II diagnosed in a 69-year-old patient with iron overload. Am J Clin Pathol. 1992;98:522-525.
40. Iolascon A, Sabato V, De Mattia D, Locatelli F. Bone marrow transplantation in a case of severe, type II congenital dyserythropoietic anaemia (CDA II). Bone Marrow Transplant. 2001;27:213-215.
41. Perrotta S, del Giudice EM, Carbone R, et al. Gilbert's syndrome accounts for the phenotypic variability of congenital dyserythropoietic anemia type II (CDA-II). J Pediatr. 2000;136:556-559.
42. Gasparini P, Miraglia del Giudice E, Delaunay J, et al. Localization of the congenital dyserythropoietic anemia II locus to chromosome 20q11.2 by genomewide search. Am J Hum Genet. 1997;61: 1112-1116.
43. Shalev H, Kapleushnik Y, Haeskelzon L, et al. Clinical and laboratory manifestations of congenital dyserythropoietic anemia type I in young adults. Eur J Haematol. 2002;68:170-174.
44. Van Steenbergen W, Matthijs G, Roskams T, Fevery J. Noniatrogenic haemochromatosis in congenital dyserythropoietic anaemia type II is not related to C282Y and H63D mutations in the HFE gene: report on two brothers. Acta Clin Belg. 2002;57:79-84.
45. Cazzola M, Barosi G, Bergamaschi G, et al. Iron loading in congenital dyserythropoietic anaemias and congenital sideroblastic anaemias. Br J Haematol. 1983;54:649-654.
46. Franchini M, Gandini G, Girelli D, Lippi G, de Gironcoli M, Aprili G. Association of phlebotomy and subcutaneous bolus injection of deferoxamine for the treatment of anemic patients with iron overload. Haematologica. 2001;86:873-874.
47. Chrobak L, Spacek J. Favorable effect of splenectomy on anemia in 3 siblings with type II congenital dyserythropoietic anemia (HEMPAS) (ultrastructural changes in erythrocytes after splenectomy) [in Czech]. Vnitr Lek. 1997;43:635-638.
48. Barosi G, Cazzola M, Stefanelli M, Ascari E. Studies of ineffective erythropoiesis and peripheral haemolysis in congenital dyserythropoietic anaemia type II. Br J Haematol. 1979;43:243-250.
49. Heimpel H, Eggl C, Griesshammer A, Maier K, Kohne E. Splenectomy in congenital dyserythropoietic anemia [abstract]. Egypt J Haematol. 2002;27:83-84.
50. Bethlenfalvay NC, Hadnagy GS, Heimpel H. Unclassified type of congenital dyserythropoietic anemia (CDA) with prominent peripheral erythoblastosis. Br J Haematol. 1985;60:541-550.
51. Bird AR, Karabus CD, Hartley PS. Unclassified type of congenital dyserythropoietic anemia (CDA) with prominent erythroblastosis. Br J Haematol. 1986;63:797-798.
52. Adams CD, Kessler JF. Circulating nucleated red blood cells following splenectomy in a patient with congenital dyserythropoietic anemia. Am J Hematol. 1991;38:120-123.
53. Fischer R, Tiemann CD, Engelhardt R, et al. Assessment of iron stores in children with transfusion siderosis by biomagnetic liver susceptometry. Am J Hematol. 1999;60:289-299.
54. Marchetti M, Quaglini S, Barosi G. Prophylactic splenectomy and cholecystectomy in mild hereditary spherocytosis: analyzing the decision in different clinical scenarios. J Intern Med. 1998;244: 217-226.