Congenital dyserythropoietic anemia type IV in the genetic era: A rare neonatal case report of rapid identification with a review of the literature

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October 6, 2022

Abstract

Congenital dyserythropoietic anemia type IV (CDAIV) is a rare inherited hematological disorder presenting severe anemia due to altered erythropoiesis and hemolysis, with variable needs for recurrent transfusions. We present a case of a transfusion-dependant male newborn who required an intrauterine transfusion and presented at birth with severe hemolytic anemia. Genetic testing rapidly identified a KLF1 gene mutation, a CDAIV variant. This case highlights the advantages of next-generation sequencing testing for congenital hemolytic anemia: diagnostic speed, guidance on natural history, and optimized clinical management and anticipatory guidance for parents and clinicians. We reviewed the literature for all CDAIV cases.

Introduction

Congenital dyserythropoietic anemia (CDA) is an aggregation of rare inherited hematological disorders characterized by a hallmark feature of altered erythropoiesis. CDA can be subdivided into 5 categories, namely type I, II, III, transcription factor related CDA and CDA variants(1). Each type is characterized by varying hematopathological findings, clinical presentation, and underlying genetic basis (1). CDAIV (Online Mendelian Inheritance in Man (OMIM): 613673) is part of the transcription factor-related CDA family and accounts for only 0.4% of all patients with CDA (1). To date, 12 patients have been identified with CDAIV (see table 1). Patients are found to have a mutation in the transcription factor KLF1, which has been implicated in various mild hematological disorders (see supplemental table 1) (2). This is likely due to the KLF1 role at multiple levels of the erythropoietic process, and varying mutations may lead to different functional consequences on the transcription factor (3-5). CDAIV is an autosomal dominant condition in which most patients harbour a *de novo* heterozygous mutation c.973G.A (p.E325K) (see table 1) (3-5). Most CDAIV patients present severe anemia, hemolysis, hepatosplenomegaly, hyperbilirubinemia, and persistence of fetal hemoglobin. They are often transiently transfusion-dependant. Many patients present in the neonatal period but are only diagnosed later in life, because of the non-specific symptomatology of hemolytic anemias.

We present a case of CDAIV, which presented with severe neonatal anemia and hyperbilirubinemia, with a subsequent rapid diagnosis through a commercially available next-generation sequencing (NGS) panel for congenital hemolytic anemias. We highlight the importance of considering early genetic testing for unexplained severe and refractory congenital anemias. Additionally, we advocate for pre-transfusion blood storage in cases of unexplained anemia in newborns to prevent delays in diagnosis due to the presence of circulating donor's RBC. Of note, we remind clinicians that it is routine practice for laboratories to store pre-transfusion blood samples for 1 week.

Case presentation

This term baby boy was born from a healthy mother with an obstetrical history notable for three early spontaneous abortions, two healthy-term babies, and one child with Axenfield Riegers Syndrome (OMIM: 180500). The prenatal course was complicated by severe fetal anemia at 26 weeks' gestation (fetal hemoglobin of 39 g/L) requiring one intrauterine transfusion. The mother's blood group was A^+. Investigations for parvovirus, toxoplasmosis and cytomegalovirus were negative. Routine serologies were protective. Non-invasive prenatal testing (NIPT), rapid aneuploidy detection and chromosomal microarray were normal. Several maternal IAT, done before and after the intrauterine transfusion, were negative. The etiology of fetal anemia remained unclear to the maternal-fetal medicine team. The patient was not referred to hematology.

The baby was born at 37 weeks GA through induced vaginal delivery for decreased fetal movement in the context of fetal anemia. Initial complete blood count (CBC) showed hemoglobin of 65 g/L with an increased MCV of 118 fL and reticulocytosis (574.2 $X10^9$/L). His blood group was A^+. The blood smear suggested hemolysis (see figure 1A). LDH was elevated at 10 446 U/L (normal < 1128 U/L). DAT and IAT were negative. He was transfused pRBC (see Figure 1B). Pre-transfusion hemoglobin investigations were not ordered. He developed early onset severe refractory unconjugated hyperbilirubinemia (see Figure 1D), requiring 3 double volume exchange transfusions in the first two days of life while under high-intensity phototherapy. He required another pRBC transfusion after the first exchange transfusion for mild anemia. He developed thrombocytopenia within the first 24h, for which he received two platelet transfusions (see Figure 1B). The thrombocytopenia resolved by DOL 4. An abdominal ultrasound showed mild hepatosplenomegaly. Given the transfused status, hemoglobin investigations and enzymopathy testing were not reliable. The Kleihauer-Betke test from the placenta was negative for feto-maternal hemorrhage. Further blood bank testing was obtained to rule out alloimmunization. Maternal serum from the time of delivery was negative for antibodies against low-frequency antigens. An eluate performed on cord blood from delivery was negative. The maternal plasma was first cross-matched against cord RBC, and then cross-matched against paternal RBC, both of which showed no agglutination. Considering the inability to test the baby's endogenous blood for hemoglobinopathies, parents were investigated with hemoglobin electrophoresis, CBC, reticulocyte and ferritin levels; all tests were unremarkable.

Concomitantly, the baby's refractory unconjugated hyperbilirubinemia progressed to significant combined hyperbilirubinemia within the first 24 hours of life (see figure 1D, E). An extensive gastroenterologic/metabolic work-up showed no primary liver disease. The conjugated hyperbilirubinemia was thought to be inspissated bile duct syndrome caused by overwhelming hemolysis. It improved with ursodiol. He was discharged on DOL 10 with no transfusion requirement for one week.

The patient required pRBCs at two weeks of age and every two weeks thereafter. At two weeks old, we performed alpha thalassemia gene mapping, which came back normal, and sent a Invitae Hereditary Hemolytic Anemia Panel (test code: 55679) genetic panel on mucosal cells. This panel uses next-generation sequencing to analyze 40 genes involved in a vast array of hereditary hemolytic anemias. At one month of age, his genetic results yielded a KLF1 gene mutation C.973G>A, a pathologic variant known to cause CDAIV. A bone marrow transplant consultation was obtained. A fully matched sibling hematologic stem cell transplant is planned once the patient is 1 year old.

Discussion

This patient presented with congenital anemia of unknown cause with a history of intrauterine transfusion requirement. Hemolytic markers were strikingly elevated. Reticulocytosis explained the high MCV. The differential diagnosis of congenital hemolytic anemia is wide (supplemental table 2). Maternal-fetal alloimmunization is the most common etiology. There are case reports of fetuses with hydrops fetalis secondary to alloimmunization that were born with a negative IAT (17). We therefore performed extensive blood bank testing that completely rule out alloimmunization. We sent a mucosal DNA NGS inherited hemolytic anemia panel. This test is not affected by the post-transfusion status. CDA was historically diagnosed by RBC property testing and bone marrow morphology. However, with the advent of genetic testing, NGS has become the gold standard to diagnose rare congenital hemolytic anemia, like CDA. The genetic basis of CDAIV was first sequenced nearly ten years ago by Arnaud & *al.* (2010), who identified two patients with

a KLF1 mutation. Twelve patients have been described since. Most show consistent features with onset in the neonatal period (8/12 patients), but delayed diagnoses occur despite accessible technologies (see table 1). We reached a diagnosis within one month of life, which is the fastest CDA diagnosis ever described. In summary, our case highlights a patient with CDA4 *de novo* mutation with *in utero*erythropoietic disturbances requiring transfusion, post-natal hemolytic anemia and combined hyperbilirubinemia transiently refractory to exchange transfusions. Thrombocytopenia has been rarely described in CDAI and CDAIV(18). The transient thrombocytopenia of this patient was considered secondary to exchange transfusions. This case demonstrates the importance of a prenatal referral to hematology and neonatology when hydrops fetalis of unknown etiology is detected for diagnostic and postnatal planning purposes. It is a reminder that it is optimal practice to obtain pre-transfusion blood sampling in an EDTA tube in patients with severe anemia. Finally, it illustrates that the use of NGS technologies can expedite early diagnosis of rare congenital anemia, like CDAIV, allows for anticipatory guidance and consequently reduces anxiety of clinicians and families, while optimizing health care utilization.

Acknowledgements

We would like to extend our gratitude to Dr. Ruth Padmore for assistance with photomicroscopy and her review of the manuscript. We want to thank the patient and their family for their inclusion in the study.

Declarations

Ethics approval, consent to participate and consent for publication

No ethics approval was required by our center for the description of case reports requiring one patient. The patient legal guardian's consented for participation and publication of this case report.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding.

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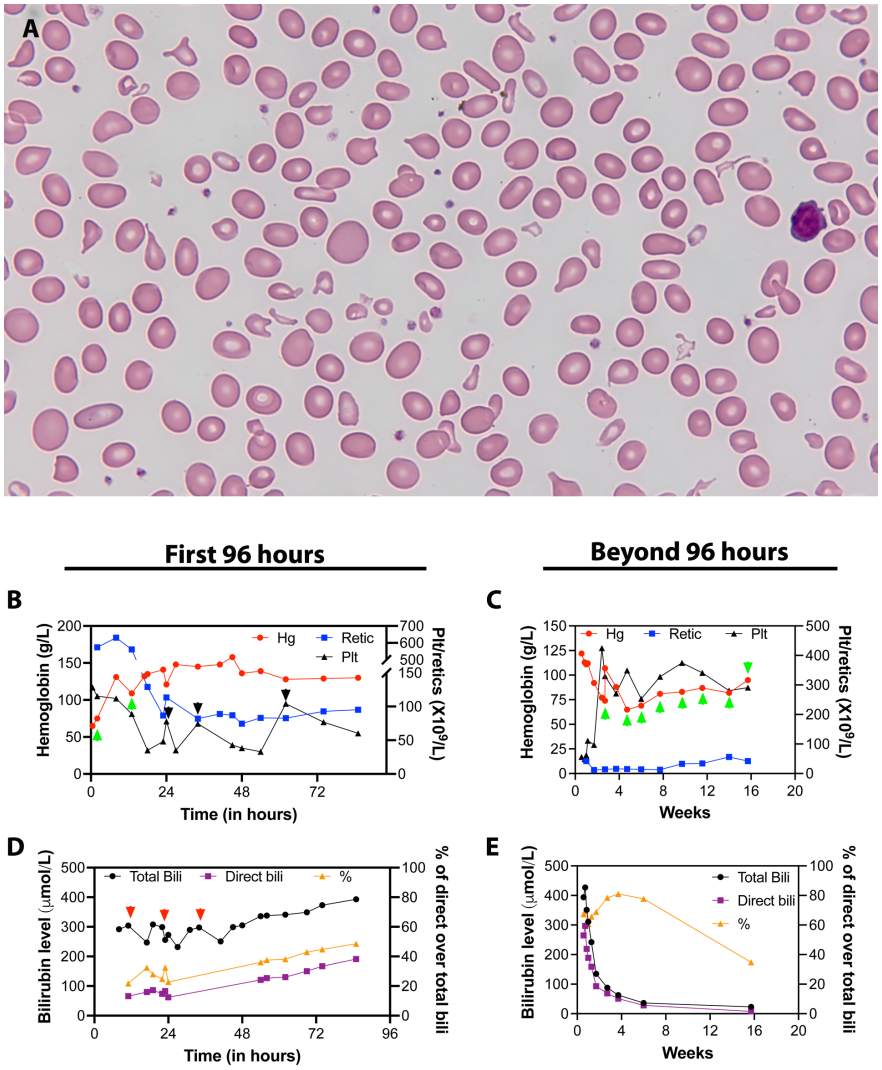
Figure 1 : **Patterns of hemoglobin, reticulocytes, platelets and bilirubin levels in the first 96 hours and onwards.** (A) Peripheral blood smear (60X) showing non-specific variable morphology: anisocytosis with microcytes, spherocytes, schistocytes, elliptocytes, macrocytes given elevated reticulocyte count, tear-drop cells, all co-occurring with transfused red blood cells of normal

appearance. (B) Early anemia & thrombocytopenia associated with brief reticulocytosis requiring

multiple blood transfusions, pRBCs transfusions (green arrowhead) and platelet transfusions (black arrowhead). (C) pRBCs transfusion (green arrowhead) requirement and hemoglobin maintenance with reticulocytopenia and rapidly resolving thrombocytopenia over time. (D) Refractory hyperbilirubinemia despite phototherapy and exchange transfusions (red arrowhead) and a progressive conjugated fraction over time. (E) Resolution of conjugated and unconjugated hyperbilirubinemia over time.

Table 1: Previous CDA4 cases described in the literature.



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