Erythrocytapheresis as a novel treatment option for adult patients with pyruvate kinase deficiency

Pyruvate kinase deficiency (PKD) is an autosomal recessive inherited disorder, caused by mutations in the *PKLR* gene on chromosome 1q21.[1] Pyruvate kinase (PK) is the second of two ATP-producing sites in the glycolytic pathway and is responsible for the conversion of phosphoenolpyruvate to pyruvate. The reduced synthesis of ATP results in inability to maintain the erythrocyte electrochemical gradient and cell membrane integrity, resulting in intra- and extravascular hemolysis.[2] PKD is the most common cause of hereditary non-spherocytic hemolytic anemia in the glycolytic pathway. Clinical manifestation of PKD varies from mild or fully compensated hemolysis, to severe hemolytic anemia already in neonatal patients, leading to anemia and jaundice, which requires treatment with phototherapy and/or exchange transfusion.[3] Associated complications are splenomegaly, jaundice, folic acid deficiency, gall bladder stones, iron overload and osteoporosis. Many patients are from childhood dependent on simple transfusions. Splenectomy removes transfusion dependency in approximately 85% of the patients.[4] Acute infections, stress and pregnancy exacerbate the anemia.[5] However, in addition to splenectomy and regular red cell transfusions treatment options are limited to iron chelation, folic acid supplement, and in a few cases allogeneic stem cell transplantation. Novel oral activators of PK and lentiviral gene therapy are in clinical trials, but may be associated with a lack of response or significant toxicity.[4,6]

Erythrocytapheresis/red cell exchange transfusion (RBCX) is often used as treatment for hyperbilirubinemia in newborns with PKD,[3] but it has never been described in adult patients with PKD. In this paper, we present two adult patients with PKD, who have received exchange transfusion to decrease their symptoms and to improve quality of life.

Both patients consented to the publication of this article.

Case 1. Debilitating fatigue: a male Caucasian patient demonstrated hemolytic anemia shortly after birth and was treated with simple and exchange blood transfusions. Due to persistent anemia in the average of 4.8–6.5 g/dL and the development of hepatosplenomegaly at the age of 2 years, a treatment with prednisolone was attempted, but with minimal response. Until the age of 3 years, the anemia was treated with simple transfusions and then a splenectomy was performed, after which transfusion independency was achieved with a hemoglobin level at 9 g/dL.

Until the age of 9 years, the cause of the hemolysis was still uncertain. The activity of PK in red cells had been measured early after birth without taking the high fraction of reticulocytes into account. A repeatedly adjusted analysis showed low activity of PK, and a pyruvate kinase isozymes R/L (*PKLR*) genotyping detected compound heterozygosity for two *PKLR* missense mutations (G364D and R510Q).

At the age of 28 years, despite transfusion independency and a hemoglobin level of >10 g/dL, the patient suffered from debilitating fatigue, muscle- and joint pain, which kept him from working and studying. In desperation due to his symptomatology, the patient changed to our clinic seeking experimental treatment options, but no clinical trials were available in Denmark.

In an attempt to both improve the hemoglobin and reduce hemolysis, the patient was offered apheresis exchange transfusion of 9-10 units of blood every 5-6 weeks. This resulted in an increased hemoglobin level (Figure 1) and less hemolysis as estimated by decreased bilirubin and lactate dehydrogenase level (Figure 1). Reticulocytosis persisted with an average of 1,070 (95% confidence interval [CI]: 650-1,489) per nL during the

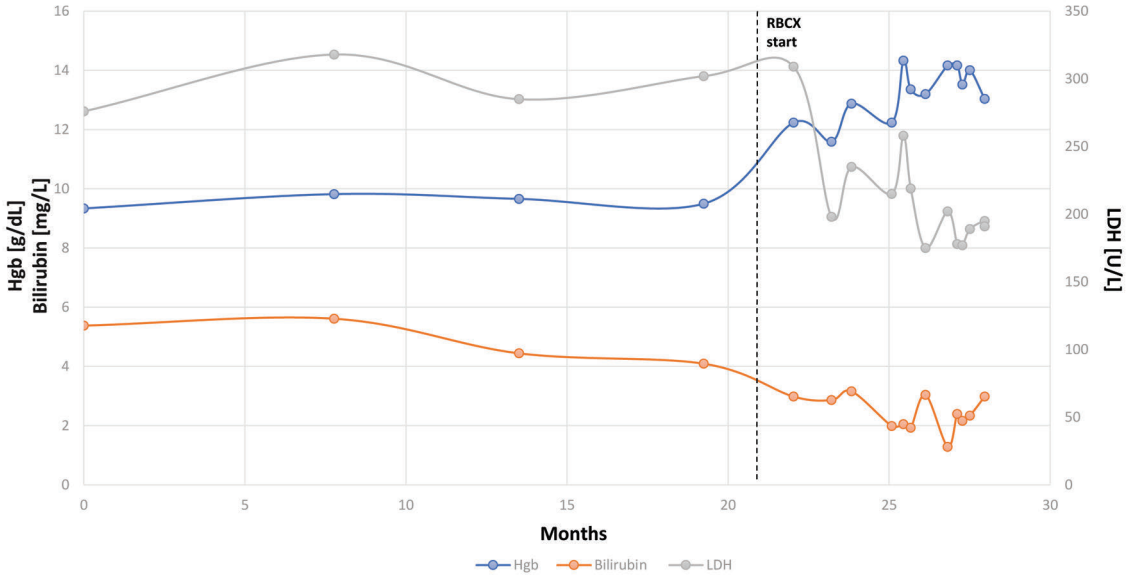

Figure 1. Case 1: Hemoglobin (Hgb), bilirubin, and lactate dehydrogenase (LDH) levels over months before and after the start of treatment with red cells exchange (RBCX) transfusions.

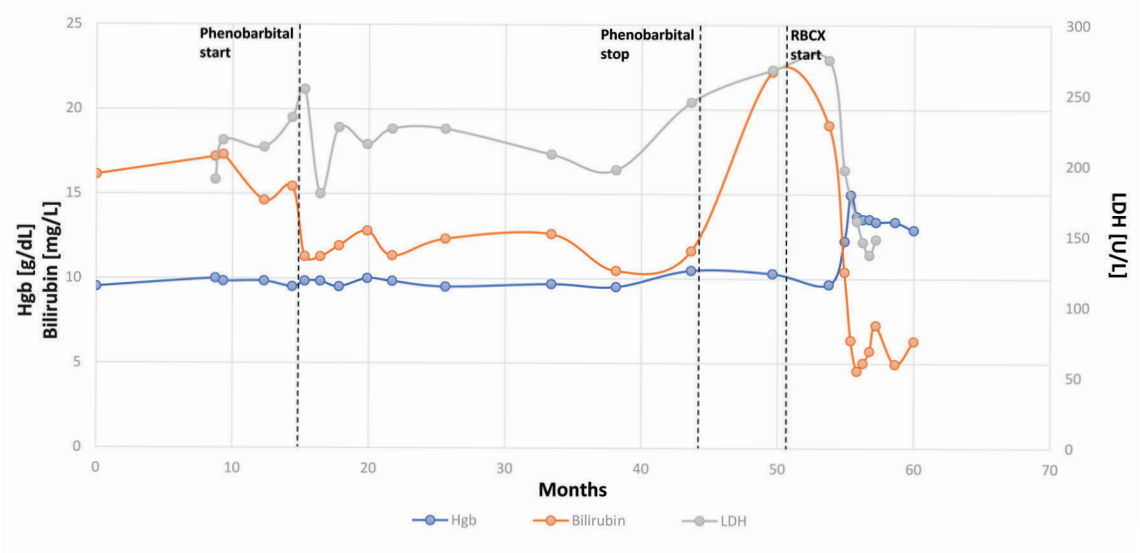

Figure 2. Case 2: Changes in hemoglobin (Hgb), bilirubin, and lactate dehydrogenase (LDH) levels over months during treatment with phenobarbital and red cells exchange (RBCX) transfusions.

year before starting RBCX and stabilized at 936 (95% CI: 728-1,144) per nL after that. Due to a good venous access status, exchange of 10 units of blood only took 1.5 hours. After the first treatment, the patient felt significantly alleviated from his fatigue and muscle- and joint pain. About a year after starting exchange transfusions, the patient was working and had lost his interest in seeking other experimental treatment options.

Case 2. Debilitating jaundice: a male Caucasian patient developed hyperbilirubinemia and hemolytic anemia shortly after birth. The hyperbilirubinemia was treated with phototherapy and exchange therapy. Tests for hemoglobinopathies, PK enzymatic assay, G6PD activity, and immune hemolysis were normal, but clouded by transfusions. At the age of 1 year, *PKLR* sequencing demonstrated compound heterozygosity for a promoter mutation (-83 G>C) and a missense mutation (R510Q). Subsequently PK enzymatic testing in the absence of transfusions confirmed very low PK activity fully compatible with the genetic findings.

The early childhood anemia was treated with regular erythrocyte transfusions until a splenectomy was performed at the age of 6 years, resulting in a stable level of hemoglobin of around 9.0 g/dL.

Nonetheless, jaundice did not improve and stayed in the range of 15-26 mg/dL. *UGT1A* sequencing demonstrated homozygosity for the common A(TA)7TAA promoter polymorphism in accordance with severe Gilberts syndrome. In late childhood and in his teenage years, the patient increasingly experienced the negative psychological and social impact of the severe jaundice. He noticed that other kids were looking at him, especially at his yellow sclerae, and he started withdrawing from social contacts. In early adulthood the patient also complained about severe fatigue. He was constantly searching for new therapeutic options such as targeted drug and gene therapy.

At the age of 17 years, off-label use of phenobarbital (15-30 mg daily) was attempted to reduce hyperbilirubinemia.[7] Over a duration of 2.5 years, bilirubin was reduced by 20-50% (Figure 2), but with little apparent reduction in skin color and – as expected – no effect on fatigue. Building on our positive experiences in sickle cell anemia, the patient was offered exchange transfusions to reduce chronic hemolysis, hyperbilirubinemia, and at the same time keep the hemoglobin at a high almost normal level.

The patient exchanged 14 units of blood every five weeks. Hemoglobin was kept above 13 g/dL and the bilirubin fell to <5.6 mg/dL (Figure 2). Reticulocytosis fell significantly from an average of 1,033 (95% CI: 885-1,181) per nL during the year before starting RBCX to 544 (95% CI: 300-787) per nL after that. Shortly after the start of exchange transfusions, the patients' phsychological burden was alleviated and the energy level was significantly increased, overall resulting in a greatly improved quality of life.

Most of our current knowledge about the clinical course of PKD is based on the PKD Natural History Study.[4] Until recently, few clinical trials existed for PKD and treatment algorithms were mostly based on experiences in transfusion dependent thalassemia. Although exchange transfusions are routinely utilized postnatally in PKD patient with hyperbilirubinemia, this option has to our knowledge not been utilized in adults before.

The two patients presented here suffered from different symptoms caused by chronic hemolytic anemia. Considering the high hemoglobin level in patient 1, his symptomatology was quite severe. This phenomenon is not rare and may be attributed to the chronic hemolysis and not the degree of anemia.[4] Patient 1 choose exchange transfusion with the goal of not only raising the hemoglobin level, but also alleviating chronic intra- and extravascular hemolysis as demonstrated by the improvement in bilirubin and lactate dehydrogenase levels. We succeeded in mitigating patient 2's jaundice to an extent neither simple transfusions nor phenobarbital could. Some bilirubin was removed by the apheresis procedure itself. When measuring total bilirubin before and within a few days after RBCX, we observed a 27.5% (95% CI: 22.9-32.2) reduction. However, unconjungated bilirubin has a short half-life in plasma, much less than a day.[8] As bilirubin remained much lower than baseline levels for weeks after the procedure, we assume that ery-

thropoiesis suppression and consequently reduction in hemolysis was the major cause of the bilirubin reduction. Similarly, the half-life of LDH *in vivo* is no more than a few days and still the LDH levels remained normalized weeks after RBCX.

In PKD, low levels of hemoglobin are often well tolerated,[4] which is often attributed to increased levels of 2,3-diphosphogylcerate shifting the hemoglobin dissociation curve rightward and thereby improving oxygen delivery.

Both patients tolerated the exchange transfusion procedure very well. Peripheral venous access was excellent, and the procedure lasted about 1.5 hours, which is nearly identical to the time spent on simple transfusions. Apheresis based RBCX should have little effect on iron balance thus limiting the need for expensive iron chelation therapy.[9] Treatment with exchange transfusion requires donor blood units, apheresis devices, and trained hospital staff. The estimated cost for one exchange transfusion procedure with 10 units of blood in Denmark is 3,160 €, and with a six-week interval the annual cost per patient is approximately 27,000 €.

Red blood cell exchange transfusion is a routinely utilized therapy in treating and preventing complications in sickle cell disease patients,[10] but carries the risk of alloimmunization. Alloimmunization risk can be minimized by extended red blood cell matching of phenotypes (ABO, Rhesus D, Rhesus CE, Kell, Kidd, Duffy and MNS),[11] which also was performed for the two described PKD patients. As both patients and most donors in Denmark are Caucasians, finding highly matched donor blood was less cumbersome than for the average sickle cell patient.

In conclusion, we present apheresis based red blood cell exchange transfusion as an attractive novel treatment option for selected patients with PKD. Not a single, serious adverse event was noted during the more than 4-year treatment practice of the PKD patients.

Rawia F.G. Jensen,[1] Morten H. Dziegiel,[2,3] Klaus Rieneck,[2] Henrik Birgens[1] and Andreas Glenthøj[1]

[1]Danish Center for Hemoglobinopathies, Department of Hematology, Herlev and Gentofte Hospital, Herlev; [2]Department of Clinical Immunology, Copenhagen University Hospital, Rigshospitalet, Copenhagen and [3]Department of Clinical Medicine, Copenhagen University, Copenhagen, Denmark

Correspondence:
ANDREAS GLENTHØJ - andreas.glenthoej@regionh.dk

doi:10.3324/haematol.2020.246686

Information on authorship, contributions, and financial & other disclosures was provided by the authors and is available with the online version of this article at www.haematologica.org.

References

1. Tanaka KR, Valentine WN, Miwa S. Pyruvate kinase (PK) deficiency hereditary nonspherocytic hemolytic anemia. Blood. 1962;19(3):267-295.
2. Mentzer WC, Baehner RL, Schmidt-Schönbein H, Robinson SH, Nathan DG. Selective reticulocyte destruction in erythrocyte pyruvate kinase deficiency. J Clin Invest. 1971;50(3):688-699.
3. Zanella A, Fermo E, Bianchi P, Valentini G. Red cell pyruvate kinase deficiency: Molecular and clinical aspects. Br J Haematol. 2005;130(1):11-25.
4. Grace RF, Bianchi P, van Beers EJ, et al. Clinical spectrum of pyruvate kinase deficiency: data from the Pyruvate Kinase Deficiency Natural History Study. Blood. 2018;131(20):2183-2192.
5. Grace RF, Mark Layton D, Barcellini W. How we manage patients with pyruvate kinase deficiency. Br J Haematol. 2019;184(5):721-734.
6. Grace RF, Zanella A, Neufeld EJ, et al. Erythrocyte pyruvate kinase deficiency: 2015 status report. Am J Hematol. 2015;90(9):825-830.
7. Perona G, Corrocher R, Frezza M, et al. Phenobarbitone sensitivity of jaundice in haemolytic patients. Br J Haematol. 1973;25(6):723-736.
8. Barrett V, Berk PD, Menken M, Berlin NI. Bilirubin turnover studies in normal and pathologic states using bilirubin-14C. Ann Intern Med. 1968;68(2):355-377.
9. Porter J, Garbowski M. Consequences and management of iron overload in sickle cell disease. Hematology Am Soc Hematol Educ Program. 2013;2013447-456.
10. Stussi G, Buser A, Holbro A. Red Blood Cells: Exchange, transfuse, or deplete. Transfus Med Hemother. 2019;46(6):407-416.
11. Raba M. Selecting red blood cell units to perform RBCX in patients with sickle cell disease. Transfus Apher Sci. 2019;58(2):142-146.