bjh review

# How we manage patients with pyruvate kinase deficiency

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## Summary

Novel therapies in development have brought a new focus on pyruvate kinase deficiency (PKD), the most common congenital haemolytic anaemia due to a glycolytic enzyme deficiency. With an improved recognition of its clinical presentation and understanding of the diagnostic pathway, more patients are likely to be identified with this anaemia. Complications, including gallstones and non-transfusion-related iron overload, require monitoring for early diagnosis and management. Current management remains supportive with red cell transfusions, chelation and splenectomy. Decisions to transfuse and/or splenectomise must be individualised. Haematopoietic stem cell transplant has been pursued in a small number of patients with mixed outcomes. Novel treatment approaches, which range from a small molecule pyruvate kinase activator to gene therapy, may transform the way in which PKD is managed in the future. In this review, we discuss the pathophysiology of PKD and present our approaches to diagnosis, monitoring and management of patients with this anaemia.

**Keywords:** Pyruvate kinase deficiency, congenital haemolytic anaemia, splenectomy, transfusions, iron overload.

To maintain its structural and functional integrity, the mature red cell has evolved a highly adapted metabolism that relies solely on glycolysis to generate adenosine triphosphate (ATP). In the resting state, at least 90% of glucose catabolism occurs anaerobically via the Embden-Meyerhof pathway. This provides not only ATP, essential for cation homeostasis, but 2,3-diphosphoglycerate (2,3-DPG) and nicotinamide adenine dinucleotide (NADH) required for modulation of haemoglobin oxygen affinity and enzymatic reduction of methaemoglobin, respectively.

Historically, the link between defective glycolysis and haemolytic anaemia emanated from observations by Selwyn and Dacie (1954) that, in patients with congenital non-spherocytic haemolytic anaemia (CNHSA), there was an increased rate of *in vitro* haemolysis after incubation of defibrinated blood that did not diminish in the presence of excess glucose. Furthermore, in some cases with this auto-haemolysis pattern (Type II), with decreased red cell ATP and increased 2,3-DPG levels, the addition of ATP reduced the rate of *in vitro* auto-haemolysis. These studies prompted Valentine and coworkers to assay the red cell activity of different glycolytic enzymes in several families with CNSHA, leading to the discovery of pyruvate kinase deficiency (PKD), which is now the most commonly recognised cause of CNSHA (Valentine *et al*, 1961). Following the original reports (Valentine *et al*, 1961; Tanaka *et al*, 1962), many more cases of PKD have been reported worldwide.

## Epidemiology

PKD exhibits autosomal recessive inheritance in nearly all cases and is widely distributed geographically. The frequency is not precisely defined, with a wide estimated prevalence of 3:1 000 000 to 1:20 000 (Beutler & Gelbart, 2000; Carey *et al*, 2000). Reported heterozygote frequencies among different populations based on qualitative and quantitative biochemical analysis range from 0·15% to 6%. Higher frequencies may reflect consanguinity or, more commonly, a founder effect. Recent data suggest the genetic epidemiology of PKD may also be influenced by selection. Evidence of a protective effect has been obtained from murine malaria models and reduced replication of *Plasmodium falciparum* in the red cells of PK-deficient patients *ex vivo* (Min-Oo *et al*, 2003; Qidwai *et al*, 2014). Phagocytosis of parasitised red cells from both heterozygous and homozygous PK-deficient individuals is enhanced (Ayi *et al*, 2008). A high frequency of PKD is found in populations in the Middle East and sub-Saharan Africa that have been subject to selective pressure from malaria (Machado *et al*, 2012; van Bruggen *et al*, 2015). PKD may be another example of genetic variation of red cell function that has evolved under the selective pressure of malaria.

## Red cell metabolism

In contrast to several of the glycolytic enzyme disorders, the clinical manifestations of PKD are, with the few exceptions noted below, confined to haemolysis and its secondary

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First published online 25 January 2019
doi: 10.1111/bjh.15758

consequences. This may be reconciled with the genetic control of the human enzyme. In its active form, PK is a homotetramer. Red cell (R) and liver (L) subunits are expressed from different tissue-specific promoters of the same gene, *PKLR*, located on chromosome 1 (1q21). Unlike mature red cells, hepatocytes retain capacity for protein synthesis, which mitigates the effects of pathogenic *PKLR* variants in the liver. Most other tissues express the muscle (M) subunit, of which there are two isoforms $M_1$ and $M_2$, encoded by a separate structural gene, *PKM*, located on chromosome 15 (15q22). During erythroid differentiation, a switch in expression takes place from the $M_2$ to the R subunit (Takegawa *et al*, 1983). In severe forms of PKD due to null mutations, reversion to $M_2$ isoenzyme synthesis may occur, rescuing a potentially lethal phenotype (Miwa *et al*, 1975).

Biochemically, PKD is heterogeneous. Typically, homozygotes exhibit <25% and heterozygotes 40–60% residual enzyme activity in red cells, though some deficient variants retain normal or near normal activity *in vitro*. PK catalyses a key step in glycolysis, the conversion of phosphoenolpyruvate (PEP) to pyruvate, which generates red cell ATP. PK-deficient reticulocytes, which generate ATP through mitochondrial oxidative phosphorylation, are at a particular disadvantage in the spleen, where limited oxygen and glucose restrict effective oxidative phosphorylation (Nathan *et al*, 1968; Mentzer *et al*, 1971). Reduced glycolytic flux *in vivo* leads to accumulation of glycolytic intermediates proximal to the PK step, particularly PEP and 2-phosphoglycerate (2-PG). Accumulation of 2,3-DPG may contribute further to impairment of glycolysis in PKD through inhibition of hexokinase. Although ATP formation is impaired in PK-deficient red cells, reduction in ATP concentration is not a consistent finding due to the high ATP content of reticulocytes.

## Diagnosis of PKD

The diagnosis of PKD is based on the presence of clinical signs and symptoms and laboratory markers of chronic haemolytic anaemia, on reduced PK enzymatic activity, and on the detection of compound heterozygous and homozygous mutations in the *PKLR* gene (Fig 1).

### *Symptoms in newborns*

*In utero* complications of affected infants are not uncommon, including intrauterine growth retardation, hydrops fetalis and prematurity (Grace *et al*, 2018a). Neonatal jaundice is frequent (59–90%), and the early neonatal course can be complicated by severe indirect hyperbilirubinaemia and significant haemolysis requiring transfusions. However, some newborns will have no evidence of jaundice and/or only mild anaemia. A blueberry muffin rash, evidence of skin extramedullary haematopoiesis, can be present at birth.

### *Symptoms in children and adults*

The most frequent symptoms are those related to anaemia (present in 90–95% of cases, from mild to transfusion-dependent), splenomegaly (80–85%, with a variable degree of enlargement), jaundice (40–70%) and gallstones (30–45%). Less common manifestations include aplastic crisis (2–14%), bone deformities (9%), extramedullary erythropoiesis (9%), delayed puberty (8%), hyperpigmentation (6%), leg ulcers and pulmonary hypertension (2–3%) (Zanella & Bianchi, 2000; Zanella *et al*, 2005; Grace *et al*, 2015, 2018a). Young children with significant anaemia may experience frontal bossing related to increased erythropoiesis and/or have an impact on their appetite and growth, requiring management with regular transfusions. The haemolysis is typically exacerbated by acute infections, stress and pregnancy. The anaemia may also be surprisingly well tolerated because of the increased red cell 2,3-DPG content, which is responsible for a rightward shift in the oxygen dissociation curve of haemoglobin. However, many patients with PKD report fatigue and low energy related to their anaemia, with a negative impact on their daily life (Grace *et al*, 2018b).

### *Laboratory findings*

Haemoglobin values generally range from 65 to 110 g/l, and most commonly improve about 15 g/l after splenectomy. Reticulocytes are usually elevated from 4% to 11% and are, paradoxically, greatly increased after splenectomy from 20% to 70% even if the anaemia becomes less severe (Nathan *et al*, 1968; Zanella *et al*, 2005; Grace *et al*, 2018b). The profound reticulocytosis is due to the longer survival of reticulocytes following splenectomy.

Red cell morphology is often unremarkable, generally displaying some degree of anisocytosis, poikilocytosis and polychromasia. A variable proportion of echinocytes (5–20%) is occasionally observed, particularly after splenectomy. The median indirect hyperbilirubinaemia is about 60 μmol/l, and higher values almost always imply coexisting Gilbert Syndrome. Ferritin is frequently increased, even in those who were not regularly transfused, and about half of patients have values over 1000 μg/l or are on chelation (van Beers *et al*, 2018; Grace *et al*, 2018a). Lactate dehydrogenase, which is mainly a marker of intravascular haemolysis, is not usually elevated in PKD (Barcellini & Fattizzo, 2015). Generally, an early onset of symptoms is associated with a severe clinical course. Overall, patients with more severe anaemia are diagnosed at an earlier age, but diagnosis may also be delayed until late adulthood (Zanella *et al*, 2005; Grace *et al*, 2018a).

### *Initial testing algorithm and differential diagnosis*

PKD is an autosomal recessive disorder; thus, the family history is typically unrevealing, with the exception of miscarriages and affected siblings. The differential diagnosis



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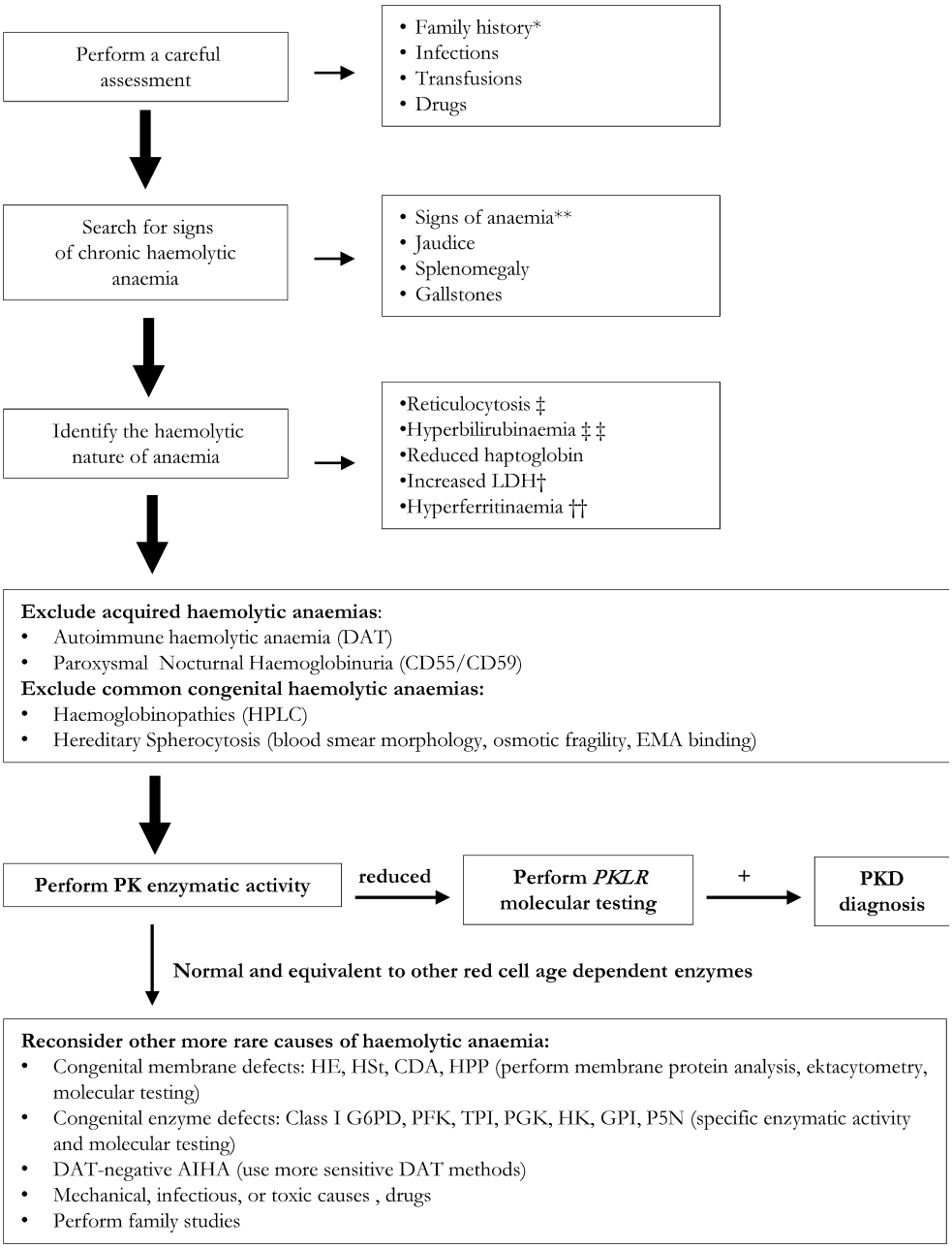


**Fig 1.** Testing algorithm and differential diagnosis of PKD. Mechanical causes of haemolysis include valvular rheumatic heart disease, infectious endocarditis, prosthetic heart valves; infectious causes include mycoplasma pneumonia, Epstein–Barr virus, cytomegalovirus, varicella, mumps, measles, Legionnaire disease, visceral leishmaniasis and secondary syphilis. Toxic causes of haemolysis include Shiga toxin-producing *Escherichia coli* and *Clostridium perfringen*. Drugs may also be responsible for immune haemolysis by a drug-independent (methyldopa, procainamide, ibuprofen, diclofenac, fludarabine and cladribine) or drug-dependent mechanism (ceftriaxone, cefotetan, penicillin, piperacillin, β-lactamase inhibitors, and other antibiotics). *Family history is absent in recessive forms and may be absent in dominant disorders as well. **Consider the co-existence of blood loss, iron and vitamin deficiencies, and liver and renal disease. ‡Reticulocytosis is also present in non-haemolytic anaemia (haemorrhage, pregnancy, acclimation) and can be inadequate in CDAs or autoimmune haemolytic anaemias with autoimmunity against reticulocytes. ‡‡Increased also in Gilbert syndrome. †Moderately increased LDH is found in cellular necrosis, tissue turnover and extravascular haemolysis. Significantly increased LDH is found in intravascular haemolysis (PNH, thrombotic microangiopathies, and prosthetic valves). ††Increased also in metabolic and inflammatory diseases, haemochromatosis and red cell transfusions. AIHA, autoimmune haemolytic anaemia; CDA, congenital dyserythropoietic anaemia; DAT, direct antiglobulin test; EMA-binding, eosin-5′-maleimide-labelled red blood cells by flow cytometric analysis; G6PD, glucose-6-phosphate dehydrogenase; GPI, glucose phosphate isomerase; HE, hereditary elliptocytosis; HK, hexokinase; HPLC, high performance liquid chromatography; HPP, hereditary pyropoikilocytosis; HS, hereditary spherocytosis; HSt, hereditary stomatocytosis; LDH, lactate dehydrogenase; P5N, pyrimidine 5'-nucleotidase deficiency; PFK, phosphofructokinase; PGK, phosphoglycerate kinase; PK, pyruvate kinase; PKD, pyruvate kinase deficiency; TPI, triose phosphate isomerase.

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includes the heterogeneous group of both the congenital and acquired haemolytic disorders (Fig 1).

### *Red cell enzyme activity*

PK enzymatic activity is usually determined in red blood cell (RBC) lysates by spectrophotometric assay. It is relatively fast, with only 2–3 h of laboratory work to perform the test, and cheap, but has several technical drawbacks, such as false normal levels due to incomplete platelet and leucocyte removal, presence of increased number of reticulocytes or recent transfusions (Table I). Moreover, kinetically abnormal mutant PK enzymes have also been described (Bianchi & Zanella, 2000; Zanella & Bianchi, 2000; Zanella *et al*, 2005). Conversely, decreased PK activity may be observed in other conditions, such as acute myeloid leukaemia and myelodysplastic syndromes. The diagnosis of PKD should be suspected when the PK enzyme activity is normal but relatively low in comparison to other age-dependent RBC enzymes, such as hexokinase or glucose-6-phosphate dehydrogenase. Importantly, there is no clear correlation between the severity of the clinical phenotype and the reduction of PK activity in the *in vitro* assay. In fact, clinically mild patients may display significantly reduced PK activity. Decreased PK activity may also be found in heterozygous carriers (Bianchi & Zanella, 2000; Zanella & Bianchi, 2000). Therefore, even when testing reveals low PK activity, genotyping of the red cell PK gene, *PKLR*, is strongly recommended to confirm the diagnosis of PKD.

### *Genetic testing*

To date, more than 300 mutations in the *PKLR* gene have been associated with PKD. The majority of these, about 70–80%, are missense substitutions, such as R510Q in Northern Europe and the United States and R486W in Southern Europe. A particularly high frequency of the R479H mutation in homozygous state exists among the Pennsylvania Amish due to a founder effect (Rider *et al*, 2011). Genetic testing offers obvious advantages compared to biochemical testing, such as the need for smaller sample volumes, no interference of transfused RBCs, suitability for prenatal diagnosis, and easier sample handling and shipping (Table I) (Gallagher & Glader, 2016). However, not all the mutations detected can be immediately classified as causative until their pathogenic nature is confirmed by other functional tests. In fact, there are patients who have normal PK activity and are homozygous or compound heterozygous for *PKLR* mutations (Zanella & Bianchi, 2000; Zanella *et al*, 2007). Therefore, both enzyme activity and genetic testing are recommended for the diagnosis of PKD. Two approaches are currently adopted: most reference centres perform PKD screening by measuring PK enzymatic activity and confirm a suspected patient with PKD by DNA analysis of the *PKLR* gene, and a few centres first sequence the *PKLR* gene by next generation sequencing panels and then confirm the diagnosis by PK enzymatic activity if a novel mutation is found (Bianchi *et al*, 2018).

### *Genotype-phenotype correlation*

With over 300 mutations described and compound heterozygous gene mutations in most patients, the relationship between the genotype and clinical symptoms in PKD is difficult to study. This topic was first investigated by describing homozygous patients, and then by studying larger series of compound heterozygous cases grouped according to anaemia severity. Severe anaemia was commonly

**Table I.** Pros and cons of enzyme *versus* genetic testing in pyruvate kinase deficiency.

| | Pros | Cons |
|---|---|---|
| Enzyme testing | • Test availability in different centres<br>• Fast (time-frame about 2 h; results available in 2–5 working days)<br>• Cheap<br>• Able to detect functional abnormality of pyruvate kinase activity | • Interference of recent transfusions (at least 40 days from the last transfusion is advisable)<br>• Reticulocytosis may give falsely normal/elevated values<br>• Leucocyte/platelet interference (need to remove buffy coat)<br>• Amount of blood required (minimum 1 ml)<br>• Inability to discriminate between heterozygous carriers and affected patients (homozygous/compound heterozygous)<br>• Shipping and storage issues (pyruvate kinase activity is considered stable for up to 14 days at 4°C)<br>• Variability of reference ranges among centres, need to include normal controls in each test, need to refer to other red cell ag-dependent enzyme activity levels (hexokinase) |
| Genetic analysis | • Requirement of small sample volume (prenatal or neonatal diagnosis)<br>• Easy handling and shipping of samples<br>• No interference of transfused red blood cell | • Time consuming and relatively expensive<br>• Need to confirm the pathogenic role of new mutations by *in silico* analysis or other functional tests<br>• May miss certain variants depending on the location and type of the genetic defect |



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associated with disruptive mutations, such as stop codon, frameshift, splicing and large deletions, and with missense mutations directly involving the active site or protein stability. Thereafter, genotypes have been investigated by the production and characterization of the recombinant mutant proteins (Zanella & Bianchi, 2000; Zanella *et al*, 2007). However, the clinical manifestations of PKD also include genetic post-translational or epigenetic modifications, along with variations in the degree of ineffective erythropoiesis and splenic function, making it complex to interpret the *in vitro* findings.

More recently, the genotype-phenotype correlation was investigated in patients enrolled in the PKD Natural History Study (Grace *et al*, 2018a). Patients were grouped according to the presence of two missense mutations (M/M), one missense/one non-missense (M/NM), or two non-missense mutations (NM/NM); non-missense mutations included nonsense, frameshift, inframe splicing mutations and other disruptive mutation variants. When compared with those patients with at least one missense mutation, the NM/NM group had a more severe phenotype, characterised by earlier diagnosis, lower haemoglobin levels, higher rate of splenectomy, greater transfusion needs and higher ferritin levels (Table II). These findings suggest that genetic testing may also be useful in discussing prognosis and establishing a monitoring plan with patients. However, the haemoglobin range overlaps among the genotype groups and the frequency of complications is high across genotypes, suggesting careful monitoring is needed in all patients regardless of the genotype.

## Complications: screening and management

### *Hyperbilirubinaemia and gallstones*

Gallstones are a frequent complication of PKD, occurring at all ages and reported in 30–45% of patients (Zanella *et al*, 2007; Grace *et al*, 2018a). The median age at diagnosis is 15 years, with 62% occurring in children <18 years old. Patients with gallstones tend to have a higher baseline bilirubin level, suggesting that, as in other haemolytic anaemias, co-inheritance of Gilbert syndrome may raise the risk of gallstones (del Giudice *et al*, 1999; Haverfield *et al*, 2005). These data also suggest that a more haemolytic pattern may contribute to gallstone development.

The timing of cholecystectomy is variable. Only 20% of patients have a simultaneous cholecystectomy and splenectomy; however, half of those who undergo splenectomy without a prior or simultaneous cholecystectomy, require a later cholecystectomy (Grace *et al*, 2018a). Therefore, we recommend regular screening by ultrasound for gallstones and prior to splenectomy (Table III). If gallstones are present, simultaneous splenectomy and cholecystectomy should be performed. In the absence of gallstones, it is less clear whether a cholecystectomy should be performed at the time of splenectomy. Given that splenectomy only partially ameliorates the haemolysis, patients remain at risk for the development of gallstones with a 50% likelihood of a future cholecystectomy after splenectomy (Grace *et al*, 2018a). On the other hand, it is possible that cholecystectomy may increase the risk of development of intrahepatic cholestasis. Therefore, the decision to perform a simultaneous cholecystectomy at the time of splenectomy in the absence of gallstones should strongly be considered, but the pros and cons should also be reviewed with the patient.

### *Iron overload*

Iron overload is a common complication of PKD (Zanella *et al*, 2005). It is a predictable complication of chronically transfused patients but is also common in patients with PKD and limited or no history of transfusions (Zanella *et al*, 1993; Marshall *et al*, 2003; Finkenstedt *et al*, 2009; van Beers *et al*, 2018). Increased intestinal iron absorption relates to both chronic anaemia and ineffective erythropoiesis. In non-transfused patients, iron loading may be due to elevated growth differentiation factor -15 levels, which contribute to low

**Table II.** Genotype-phenotype associations in 193 patients with Pyruvate Kinase deficiency.

| | NM/NM<br>*N* = 29 | M/NM<br>*N* = 52 | M/M<br>*N* = 111 | *P*-value |
|---|---|---|---|---|
| Age at diagnosis (years, median, range) | 0·4 (0 to 10·9) | 0·7 (0 to 42·3) | 1·3 (0 to 60·3) | 0·049 |
| Haemoglobin* (g/l, median, range) | 79 (65 to 89) | 84 (64 to 128) | 92 (43 to 123) | 0·003 |
| Transfusions (total lifetime median number, range) | 65 (3 to 991) | 25 (1 to 721) | 16 (1 to 1915) | 0·0015 |
| Rate of splenectomy at age ≥5 years (number of patients, %) | 21/29 (72) | 26/52 (50) | 49/111 (44) | 0·0001 |
| Maximum ferritin (μg/l, median, range) | 1787 (423 to 13409) | 604 (22 to 8220) | 573 (31 to 9679) | <0·0001 |
| Iron overload** (number of patients, %) | 21/25 (84) | 20/38 (53) | 33/77 (43) | 0·0013 |
| Normalised PK enzyme activity (% of normal, median, range) | −41·6 (−152 to 15) | −51·9 (−211 to 64) | −69·6 (−486 to 118) | 0·16 |

Adapted from Grace *et al* (2018a). M/M, two missense mutations; M/NM, one missense/one non-missense mutation; NM/NM, two non-missense mutations; PK, pyruvate kinase.

*Haemoglobin levels are given for the splenectomised, not regularly transfused cohort. Regular transfusions are defined as ≥6 transfusions within 12 months.

**Iron overload is defined by maximum ferritin >1000 μg/l or received chelation in prior 12 months.

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hepcidin levels and increased iron absorption; however, this observation has been inconsistent (Finkenstedt *et al*, 2009; Rider *et al*, 2011). Although co-inheritance of haemochromatosis-related genetic defects may contribute to the risk of iron loading, this does not appear to be the main explanation for iron loading in this patient population.

In the PKD Natural History Study, iron overload, as defined by ferritin >1000 μg/l, was present in 38% of the patients who were not receiving regular transfusions (van Beers *et al*, 2018). By using T2* magnetic resonance imaging (MRI), the rate of iron overload, defined as liver iron concentration (LIC) >3 mg Fe/g dry weight liver (DW), in non-regularly transfused patients with PKD, rose to 82%. Cardiac iron overload, defined as a cardiac T2* ≤ 20 ms, was also demonstrated in 7% of cases. In this cohort, 80% of patients were not regularly transfused, but many had received transfusions historically and about one third were periodically on chelation for a median of 5 years. Therefore, it is difficult to assess the real presence of iron overload independent from transfusion support and chelation therapy, which are both intermittent, and based on physicians' attitude and patients' preferences. However, 10% of those who had never been transfused had received chelation, and 6 of 7 never transfused patients met the MRI criteria for iron overload. These

**Table III.** Recommended monitoring for patients with PKD*.

| Condition to monitor | Recommended monitoring |
|---|---|
| Haemolytic anaemia | • Check complete blood counts, reticulocyte count, bilirubin at least yearly, frequency depends on acute stressors and transfusion needs. |
| Gallstones | • Screen annually by ultrasound for gallbladder disease and prior to splenectomy. If gallstones are present, a cholecystectomy should be performed.<br>• If gallstones are found prior to splenectomy, then a simultaneous splenectomy and cholecystectomy should be performed. In the absence of gallstones, the decision to perform a simultaneous cholecystectomy should be considered and discussed with the patient reviewing the pros and cons. |
| Aplastic crisis | • In patients with reticulocytopenia, we recommend checking a parvovirus IgM or PCR once to confirm diagnosis.<br>• Educate patients with PKD about the risk of an aplastic crisis with parvovirus and need for close monitoring after exposure. |
| Blood-borne viral Infections | • Screen for HIV and hepatitis viruses annually in patients who have received transfusions in the prior year |
| Iron overload | • Evaluate ferritin levels annually (in non-transfused), at least twice yearly in transfused patients, and every 1–3 months while on chelation.<br>• Evaluate annual T2* MRIs for patients on regular transfusions. In non-regularly transfused patients, consider first study once sedation is unnecessary, particularly in patients diagnosed with PKD at a young age, with severe haemolysis, and/or ferritin >500 μg/l. The frequency of MRI should be based on the initial findings and correlation with ferritin. |
| Osteopenia | • Evaluate 25, hydroxy-vitamin D levels. Consider monitoring with DEXA scans for baseline assessment in adolescence or early adulthood. The frequency of follow-up monitoring depends upon the findings on the initial assessment. |
| Endocrinopathies | • Screen for endocrinopathies annually in individuals with iron overload, including thyroid dysfunction, sex hormone abnormalities and diabetes. Note that a haemoglobin A1C will not be accurate in the setting of haemolytic anaemia; therefore, screening fructosamine levels is recommended for screening for diabetes. |
| Pulmonary hypertension | • Consider an echocardiogram in individuals aged >30 years, prior to pregnancy, and at any age in individuals with symptoms concerning for poor cardiac function and/or pulmonary hypertension |
| Extramedullary haematopoiesis | • Image based on the development of new concerning symptoms |

DEXA, dual-energy X-ray absorptiometry; HIV, human immunodeficiency virus; MRI, magnetic resonance imaging; PCR, polymerase chain reaction; PKD, pyruvate kinase deficiency.

*The types and frequency of necessary tests for monitoring may differ between patients. Additional medications, such as iron chelation, or co-morbid conditions will require additional monitoring.



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findings reinforce the hypothesis that iron overload in PKD is multifactorial in its pathogenesis with aetiologias that may include chronic haemolysis, increased iron turnover, ineffective erythropoiesis, splenectomy and haemochromatosis mutations, even in the heterozygous state.

*Iron monitoring in regularly transfused patients.* In patients receiving 6 or more transfusions per year, we recommend regular monitoring of ferritin levels, at least twice yearly. In patients receiving chelation therapy, ferritin measurements should be followed more frequently. After 10–20 transfusions, iron overload is present and can lead to increased circulating free iron, which is highly toxic (Porter, 2001; Hoffbrand *et al*, 2012). Once patients have received approximately 10–14 red cell transfusions, if available, an MRI for iron measurement should be obtained and followed on an annual basis while patients are chelated.

*Iron monitoring in non-regularly transfused patients.* In patients with PKD, ferritin levels of >1000 μg/l have a sensitivity for LIC > 3 mg/g DW of only 53%, whereas the sensitivity of a ferritin cut-off of 500 μg/l is 90% (van Beers *et al*, 2018). Based on the high incidence of non-transfusion related iron overload in PKD, we recommend regular monitoring of ferritin levels, at least every 1–2 years. If MRI assessment of iron overload is available, non-regularly transfused patients should have an MRI once the patient is at an age in which the MRI can be conducted without sedation. An MRI is indicated in patients diagnosed with PKD in the first several months of life, with severe haemolysis, and/or with a ferritin >500 μg/l; however, iron overload is common, and many patients have significant iron loading in the absence of clear predictors.

*Iron management.* Chelation therapy is necessary in regularly transfused patients and may be required intermittently in non-transfused patients with PKD as MRI studies are monitored. The approach to chelation in PKD is similar to other red cell and iron loading disorders. In non-transfused patients with adequate haemoglobin levels, therapeutic phlebotomy can be considered as an alternative to chelation. However, phlebotomy has only been attempted in the minority of patients (<3%) and may be both less effective as compared with chelation and not well tolerated in significantly anaemic or symptomatic patients. If phlebotomy is attempted, a small volume, 50–100 ml in an adult, should be removed at the first visit with increasing volumes over time, up to 400 ml, as the patient's ability to tolerate this approach is assessed.

### *Aplastic crisis*

The clinical course may be complicated by an aplastic crisis, characterised by an abrupt onset of profound anaemia and reticulocytopenia, often associated with a variable degree of pancytopenia. These crises usually are related to infection with parvovirus B19, which is cytotoxic for erythroid progenitors. An aplastic crisis may be the initial manifestation of several hereditary haemolytic anaemias and may cause confusion at the initial diagnosis. Most patients will require a blood transfusion during an aplastic crisis. Generally, recovery occurs in about 10 days, but anaemia lasting more than 1 month has also been observed in some cases. We recommend educating patients with PKD about the risk of an aplastic crisis with parvovirus and close monitoring after known exposure. In patients with reticulocytopenia, we recommend checking a parvovirus IgM or polymerase chain reaction to confirm the diagnosis. In patients with PKD, an aplastic crisis from an acute parvovirus infection occurs only once in a lifetime.

### *Haemolytic crises*

Haemolytic episodes or crises develop in the setting of stressors or triggers of haemolysis, which most often are due to infections and, thus, are more frequent in childhood. Pregnancy can also be a common haemolytic trigger. Symptoms include increased fatigue, pallor, scleral icterus and jaundice associated with severe anaemia, indirect hyperbilirubinaemia and haemoglobinuria. The spleen size may also increase during haemolytic episodes. Patients may need transfusion support during these episodes despite a marked reticulocytosis.

Daily folic acid supplementation may be beneficial to support erythropoiesis in patients with moderate haemolysis associated with a reticulocytosis >15%. In patients with mild haemolysis but a limited diet, folic acid can be supplemented intermittently. Although this has not been well studied in PKD, folic acid supplementation may be important during haemolytic crises, pregnancy and in childhood during growth and development.

### *Splenomegaly*

Splenomegaly is present in 80–85% of individuals with PKD. Hypersplenism can occur in the setting of splenomegaly and should be suspected in patients with an increasing transfusion burden and/or mild thrombocytopenia or neutropenia. Individuals with palpable splenomegaly may be at risk for splenic injury with trauma to the abdomen. Although splenomegaly is typical, a normal spleen size does not exclude PKD or the role of the spleen in the patient's haemolysis.

### *Osteopenia*

Bone changes associated with a hyperplastic bone marrow occasionally may result in frontal bossing. Patients with PKD are at risk for low bone mineral density, fractures and bone pain. Bone disease is a common complication of thalassaemia syndromes due to marrow expansion, iron toxicity and its

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treatment, increased bone turnover and hormone deficiencies (Vogiatzi *et al*, 2009; Wong *et al*, 2016; Nakavachara *et al*, 2018). Other haemolytic disorders, such as sickle cell anaemia and autoimmune haemolytic anaemia, have similarly shown an effect on bone health (Steer *et al*, 2017). The mechanisms leading to osteopenia in patients with PKD are much less clearly understood but may be similar. Close attention to vitamin D and calcium intake may be beneficial. Given that the mechanism for osteopenia in PKD is poorly understood but the risk probably increases with age, we recommend consideration of monitoring with dual-energy X-ray absorptiometry (DEXA) scans for baseline assessment in late adolescence or early adulthood. The frequency of follow-up monitoring depends upon the findings on the initial assessment.

### *Other complications*

Extramedullary haematopoiesis, driven by ineffective erythropoiesis, occurs in about 10% of patients (Grace *et al*, 2018a). This occurs most commonly in the liver and spleen but also occurs in the paravertebral area and mediastinum (Aizawa *et al*, 2003; Plensa *et al*, 2005). Rarely, extramedullary haematopoiesis occurs in the central nervous system, eye, lymph nodes, lung or pleura. These lesions are diagnosed by radiological imaging and/or a tissue biopsy. Pulmonary hypertension, diagnosed by echocardiogram and confirmed with cardiac catheterization, is a complication in 3% of patients with PKD post-splenectomy (Bachmeyer *et al*, 2009; Grace *et al*, 2018a). The aetiology in PKD is not clear but, given this well-described complication in other haemolytic anaemias, it probably relates to ongoing haemolysis. This diagnosis should be investigated in patients with unexplained shortness of breath. Also, as with other haemolytic anaemias, leg ulcers have been reported in PKD (Muller-Soyano *et al*, 1976; Grace *et al*, 2018b). The aetiology is multifactorial but not well studied in this anaemia. We recommend skin examination at routine visits.

## Supportive treatments

### *Role of transfusions*

*Neonatal hyperbilirubinaemia and transfusions.* Neonatal indirect hyperbilirubinaemia is treated with phototherapy (93%) and/or exchange transfusions (46%) (Grace *et al*, 2018a). Phototherapy is effective in preventing exchange transfusions in many infants. Rarely, newborns can present with evolving hepatic disease and evidence of a direct hyperbilirubinaemia (Raphael *et al*, 2007; Olivier *et al*, 2015; Chartier *et al*, 2018). One hypothesis of the pathophysiology for this uncommon complication is that certain patients are susceptible to PK-liver (PK-L) enzyme deficiency due to both severe deficiencies in PK-L and the PK-M2 isoenzyme. In the setting of deficiency of both enzyme subtypes, hepatocytes become deficient in ATP, leading to hepatocellular injury. In published cases, there is a high rate of morbidity and mortality. The only reported effective strategy to date is early identification of the aetiology of liver disease followed by liver transplant (Chartier *et al*, 2018).

Many newborns will develop significant anaemia in the first few days of life requiring transfusion. Assessment of the haemoglobin levels is challenging due to suppressed erythropoiesis from early transfusions combined with the physiological haemoglobin nadir. After the initial transfusion, we recommend attempting to extend the time between transfusions to allow erythropoietin to drive reticulocyte production and assessment of the baseline haemoglobin. Allowing the infant's haemoglobin to drop may lead to a rise in reticulocytes and, then, a rise in haemoglobin to the patient's non-suppressed baseline.

*Transfusions in children and adults.* The decision to transfuse a patient with PKD relates to the patient's tolerance of anaemia rather than an arbitrary level of haemoglobin (Table IV). Because of increased red cell 2,3-DPG content, patients may tolerate moderately severe anaemia with few symptoms due to enhanced oxygen unloading from haemoglobin. Unlike thalassaemia, in PKD, there is currently no data to support a strategy of transfusing to keep the haemoglobin above a set nadir with a goal of avoiding complications. This strategy exposes patients to risks without clear benefit. Rather, transfusions should be individualised, based on the patient's symptoms, level of activity, and assessment of the impact of the anaemia on their quality of life.

As individuals with PKD age, haemoglobin levels that were well tolerated at a younger age may no longer be tolerated. Furthermore, only after a receiving a transfusion do some patients appreciate that they had unrecognised significant symptoms of anaemia. The haemoglobin level in patients with PKD is generally stable over time. Patients who have worsening of their anaemia of unclear cause, should undergo investigation for nutritional deficiencies, evolving myelodysplasia or marrow infiltration, or growth of an accessory spleen.

Most individuals with PKD (84%) will have been transfused at least once in their lifetime. Despite this frequency, allo-sensitisation is relatively uncommon, occurring in only 3% of patients. During the first years of life, haemoglobin goals are those that allow for normal growth and development. Some children are only intermittently transfused for symptoms associated with haemolysis with viral infections. Other children are transfused at regular intervals to avoid daily symptoms of anaemia. The interval for these transfusions is based on a goal to keep the haemoglobin nadir from dropping below the threshold at which patients develop symptoms. For most individuals with PKD, transfusion needs decrease after splenectomy. Transfusions become less common with age, probably relating both to the timing of splenectomy and the frequency with which patients develop viral infections that cause increased haemolysis. About half of the patients aged $\leq$5 years old receive $\geq$6 transfusions per


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**Table IV.** Recommended supportive management for patients with PKD.

| Supportive management | Recommendations |
|---|---|
| Diagnosis of PKD | • PKD should be diagnosed by both (1) a reduced PK enzyme activity (compared to other red cell age-dependent enzymes) and (2) the detection of compound heterozygous or homozygous mutations in the *PKLR* gene. PKD should be suspected in patients at any age with chronic haemolytic anaemia. |
| Folic acid supplementation | • Daily folic acid supplementation may be beneficial to support erythropoiesis in patients with moderate haemolysis associated with a reticulocytosis >15% or in patients with mild haemolysis but a limited diet.<br>• Folic acid supplementation may be important during haemolytic crises, pregnancy, and in childhood during growth and development. |
| Red cell transfusions | • Transfusions should be individualised based on the patient's tolerance of anaemia, level of activity, and quality of life, rather than on an arbitrary level of haemoglobin.<br>• After an initial transfusion, extend the time between transfusions if the patient is relatively asymptomatic and, in children, with good growth.<br>• In patients who have worsening of their anaemia in the absence of a clear haemolytic trigger, further investigation should include nutritional deficiencies, evolving myelodysplasia or marrow infiltration, or growth of an accessory spleen. |
| Full splenectomy | • *Indications*: (1) Patients with PKD who receive regular transfusions or are severely anaemic, although these cases are least likely to have a haemoglobin response; (2) Patients with massive splenomegaly at risk of spleen rupture due to lifestyle choices.<br>• *Timing of splenectomy*: Typically deferred until after the age of 5 years but not later than late childhood/adolescence.<br>• *Type of splenectomy*: We do not recommend partial splenectomy.<br>• *Post-splenectomy sepsis*: Patients should be educated about immunizations, prophylactic antibiotics, the risks of post-splenectomy sepsis, and the proper conduct in case of fever >38·5°C.<br>• *Post-splenectomy thromboprophylaxis*: Prophylactic anticoagulation can be considered, once safe from a bleeding perspective, immediately post-splenectomy, in those with other thrombotic risk factors. Low dose aspirin could be considered until the platelet count is $<500 \times 10^9$/l in adults with advanced age, a history of thrombosis, hypercholesterolaemia and cigarette smoking. Low dose aspirin can be considered in children until the platelet count is $<1000 \times 10^9$/l. |
| Stem cell transplant | • We do not recommend bone marrow transplantation for PKD based on available data on outcomes with current approaches. |
| Management in pregnancy | • Multidisciplinary care with a haematologist and high-risk obstetrician with close attention to fetal growth and transfusions to the pregnant woman on the basis of both her symptoms and fetal ultrasounds/monitoring. |

PK, pyruvate kinase; PKD, pyruvate kinase deficiency.

year, whereas only one quarter of patients aged >5 to <12 years and less than 10% of those aged ≥18 years receive regular transfusions (Grace *et al*, 2018b).

### *Management of PKD in pregnancy*

Pregnancy in women with PKD has been associated with good maternal and fetal outcomes (Fanning & Hinkle, 1985; Wax *et al*, 2007; Grace *et al*, 2018a). Fertility in women with PKD appears similar to the general population. Preterm births occur in about 10% of women, and 18% of pregnancies result in a miscarriage. Prior to conception, women who have been previously transfused should be screened for hepatitis B and C and human immunodeficiency virus (HIV). A preconception echocardiogram could also be considered to evaluate cardiac function due to chronic anaemia and risk of iron overload and pulmonary hypertension. Prior to conception, folic acid supplementation should be prescribed. Prenatal vitamins containing iron and any other iron supplement during pregnancy should be avoided unless there is documentation of true iron deficiency through laboratory iron testing.

Multidisciplinary care with a haematologist and high-risk obstetrician is recommended with close attention to fetal growth. Some obstetricians recommend monthly ultrasounds to assess fetal growth after 20 weeks and biophysical profiles by at least 32 weeks (Wax *et al*, 2007). During pregnancy, the degree of haemolysis typically worsens and transfusion needs increase. The majority of women will be transfused

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during the pregnancy or after the delivery regardless of transfusion status prior to pregnancy. Given the rarity of information about PKD in pregnancy, we do not have specific recommendations with regard to a haemoglobin threshold for transfusion during the pregnancy. However, we strongly consider transfusions and a higher haemoglobin nadir to allow for normal fetal growth. Maternal-fetal iron delivery helps to balance the iron loading that is associated with transfusions during the pregnancy.

### *Splenectomy*

Splenectomy partially ameliorates the anaemia in the majority of patients with PKD and can be beneficial in decreasing transfusion requirements. However, because of the well-known risk of post-splenectomy sepsis due to encapsulated organism bacteraemia in young children, surgery is typically delayed until 5 years of age, whenever possible. In determining the timing of splenectomy, one must weigh the risk of post-splenectomy sepsis with the risks of red cell transfusions and iron loading. In addition to considering the infectious risk, the timing of splenectomy depends on many factors, including, but not limited to, whether chelation therapy is effective or tolerated in terms of toxicity, adherence and cost, whether allo-sensitisation has developed, and whether the patient has challenging venous access. Preoperative assessment of red cell survival, splenic sequestration, and/or spleen size is of no value in selecting patients for splenectomy, and this partly reflects the importance of the liver as a site of red cell destruction. Splenectomy most commonly is followed by improvement, but not a complete correction, of the haemolysis (Keitt, 1966; Nathan *et al*, 1968; Tanaka & Paglia, 1971). Transfusion requirements, if present before splenectomy, decrease or are eliminated in 90% of patients, with a median rise in haemoglobin of 16 g/l. In almost all patients, an incompletely compensated haemolytic process with reticulocytosis and indirect hyperbilirubinaemia persists. Given the persistence of haemolysis and indirect hyperbilirubinaemia after splenectomy, it is important to advise patients that the risk of pigmented gallstones persists and the splenectomy will not resolve jaundice.

The majority of older children and adults with PKD have had a splenectomy. The decision to proceed with splenectomy appears more common in certain centres, whereas in other centres, patient remains on a transfusion and chelation schedule with their spleens intact (Table V). Many patients proceed with splenectomy to improve anaemia, decrease transfusion burden and improve health-related quality of life (Grace *et al*, 2018a). Recent guidelines have recommended splenectomy for patients with PKD who receive regular transfusions or are severely anaemic (Iolascon *et al*, 2017). Predictors of a haemoglobin response to splenectomy, defined as a haemoglobin ≥80 g/l, include a higher pre-splenectomy haemoglobin, two missense *PKLR* mutations and a lower total bilirubin level. Thus, patients with the most severe haemolysis and anaemia are least likely to respond to splenectomy.

Few cases of partial splenectomy in PKD have been reported. In the 3 patients reported, the haemoglobin response was relatively poor, with a post-procedure haemoglobin <80 g/l and/or continued transfusion burden (Sandoval *et al*, 1997). Therefore, we recommend full splenectomy rather than partial.

**Table V.** Potential risks and benefits of full splenectomy *versus* regular transfusions in pyruvate kinase deficiency.

| | Benefits | Risks |
|---|---|---|
| Splenectomy | • Partially ameliorates the anaemia in the majority of patients. Raises the haemoglobin a median of 16 g/l, which is a stable increase outside of infections and other stressors. Decreases burden of transfusion in 90% of patients. | • Only partial improvement in anaemia/haemolysis, so risks of haemolysis remain, including aplastic crisis, gallstones and iron loading<br>• Up to 10% of patients have no improvement in haemoglobin or haemolysis; typically, in patients with more severe haemolysis pre-splenectomy<br>• Risk of post-splenectomy sepsis with encapsulated organisms, even with perfect adherence with vaccinations and prophylactic antibiotics, and other rare infections (malaria, babesia)<br>• Risk of post-splenectomy venous thrombotic events is 10%; risk may be higher if diagnosis of pyruvate kinase deficiency is incorrect. |
| Regular transfusions | • Increase in haemoglobin without the associated risks of splenectomy. | • Impact of regular transfusions on quality of life, including symptoms associated with the nadir haemoglobin and the impact of time being transfused<br>• Need for regular intravenous access<br>• Substantially increased risk of iron loading with associated need for lifelong chelation<br>• Risk of allo-sensitisation; appears as low as 3%<br>• Risk of blood-borne infections |


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*Post-splenectomy sepsis.* Splenectomy increases the susceptibility to serious bacterial infections with encapsulated organisms and other organisms, such as malaria and babesiosis (Zahid & Bains, 2017). With adequate vaccinations and prophylactic antibiotics, the absolute risk of a serious infection is very low. Given that the spleen is the primary site for the production of IgM antibodies required for opsonization of encapsulated organisms, vaccination at least 2 weeks prior to splenectomy is essential. Vaccination schedules are regularly updated based on new information and vaccine development. Therefore, physicians should refer to an updated website, such as the vaccine recommendations for asplenic patients from the Centers for Disease Control and Prevention, for a list of vaccinations for asplenic patients.

Oral antibiotics for infection prophylaxis are indicated after splenectomy. The ideal duration for prophylactic antibiotics is not clear. Some physicians recommend a lifetime of prophylactic antibiotics, and others will recommend discontinuation after 1 year if patients live close to a medical centre and agree to seek urgent medical care for all fevers. We recommend that asplenic children receive daily prophylaxis with penicillin VK until at least 5 years of age and for at least 1 year following splenectomy. Patients should be educated that a post-splenectomy fever is a lifelong risk which requires urgent assessment within an hour, laboratory evaluation and broad spectrum antibiotics. In a large cohort of patients with PKD, post-splenectomy sepsis was higher than expected, occurring in 7% of patients (Grace *et al*, 2018a). It remains unclear whether these patients were up to date on their immunizations or taking prophylactic antibiotics.

*Post-splenectomy thrombosis.* Prior to splenectomy, the risk of thrombosis does not appear increased in individuals with PKD. Splenectomy, in general, is associated with an increased risk of thrombosis, even in otherwise healthy individuals (Kristinsson *et al*, 2014; Lin *et al*, 2016). After splenectomy, the overall risk of thrombosis in PKD is approximately 10%, including portal vein thrombosis, deep vein thrombosis, pulmonary embolism and central nervous system thrombosis (Grace *et al*, 2018b). Once cleared from a bleeding perspective, prophylactic anticoagulation after splenectomy could be considered in patients with increased thrombotic risk factors. Reactive thrombocytosis is a predictable finding after splenectomy. To prevent the cardiovascular risk, low dose aspirin can be considered until the platelet count is $<500 \times 10^9$/l in those patients with advanced age, a history of thrombosis, or hypercholesterolaemia. A higher platelet count threshold, $<1000 \times 10^9$/l, is often considered in children.

### *Stem cell transplant*

Haematopoietic stem cell transplant (HSCT) can cure PKD. However, the clinical criteria for transplantation are not clear. HSCT has been used to correct severe haemolytic anaemia associated with PKD in basenji dogs, and marrow transplantation has also been shown to be effective in mice with splenomegaly and chronic haemolytic anaemia due to PKD (Weiden *et al*, 1976; Morimoto *et al*, 1995). These animal studies have been followed by several reports of human HSCT for the treatment of PKD (Tanphaichitr *et al*, 2000). There has been a global effort to obtain the results of all patients treated with HSCT, and a report of 16 patients with PKD who underwent transplant in Europe and Asia has been published (van Straaten *et al*, 2018). In this cohort, a range of conditioning regimens and management strategies was used; the results indicate a 74% cumulative survival but also a high rate of graft-versus-host disease (GVHD), particularly in patients who were more than 10 years old when they were treated. Given the varying donor types, conditioning, GVHD and infection prophylaxis, the most effective and safest transplant regimen in patients with PKD is not yet clear.

Despite the reported success of HSCT in several patients, given the available data regarding the risks of transplant and the long-term course of PKD, we recommend an approach of splenectomy and/or regular red cell transfusions rather than HSCT. There are no currently open trials of HSCT for PKD. As the approach to the conditioning regimen is refined and the risk of mortality and morbidity decrease, assessment of the risk-benefit ratio may shift.

## Potential for a changing treatment landscape

The current management approach for patients with PKD is supportive. Therapies currently in clinical development, including gene therapy and an oral PK activator, have the potential to directly impact PK expression and activity and both extend the red cell lifespan and decrease haemolysis. If these therapies become clinically available, the approach to managing PKD would be significantly altered and improved.

### *Oral pyruvate kinase activator*

A pharmacological, small molecule, oral activator of red cell pyruvate kinase, AG-348, is currently in clinical trials (NCT02476916, NCT03548220, NCT03559699). This drug activates wild-type PK *in vitro* as well as mutant PK across a wide spectrum of variants. In preclinical studies, the drug activates PK and enhanced glycolytic flux in mice and patient samples *ex vivo* (Kung *et al*, 2017). In healthy individuals, the drug induces PK activity *in vivo*, increases red cell ATP and decreases 2,3-DPG (Yang *et al*, 2018). In the interim results from a phase II clinical trial in non-transfused patients with PKD receiving twice daily dosing, approximately half of the patients had a maximal increase in haemoglobin of greater than 10 g/l (median 34 g/l) (Grace *et al*, 2017). In the patients with a haemoglobin response, the haemoglobin level was sustained over the remainder of the core period of the study and, in those who continued on drug, into the extension study. These patients also had evidence of increased flow through the glycolytic pathway as well as

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improvement in haemolytic markers, including an associated decrease in reticulocyte count and indirect bilirubin and an associated increase in haptoglobin. A correlation between genotype and haemoglobin response was seen, in which patients with at least one missense mutation were more likely to have a haemoglobin response. AG-348 was generally well-tolerated in the phase II trial with the majority of adverse events of Grade 1 or 2, including, most commonly, headaches, insomnia and nausea. Several serious adverse events were reported, including haemolysis with abrupt drug withdrawal, hypertriglyceridaemia and osteoporosis. AG-348 has been noted to be a reversible mild aromatase inhibitor, and the trials have been limited to the adult population to date. The enrolling phase III study will determine whether this oral activator is an effective and safe treatment for adults with PKD. Long-term follow-up in patients on this agent will determine whether the rise in haemoglobin is also associated with a decreased incidence of complications.

## Gene therapy

Because PKD is the result of a defect in a single gene and affects primarily one cell type, it is also considered a potential candidate disease for gene therapy. The feasibility of gene therapy for PKD was first demonstrated in mouse models, with a preclinical study using a lentiviral vector in myeloablated PK-deficient mice demonstrating a post-induction increase in PK activity and haemoglobin and a reduction in reticulocyte count and spleen size (Tani *et al*, 1994; Kanno *et al*, 2007; Meza *et al*, 2009; Garcia-Gomez *et al*, 2016). Metabolic studies in these mice following therapy were consistent with improved flow through the glycolytic pathway. Based on these data, future trials of gene therapy for PKD are planned. Given the genotype-haemoglobin response relationship seen with AG-348 in the phase II clinical trial, patients with two loss of function or non-missense *PKLR* variants are not eligible for current phase III studies of AG-348 but will probably be eligible for trials of gene therapy for PKD.

## Summary

Understanding the clinical symptoms and presentation of patients with haemolytic anaemias and the diagnostic pathway for PKD is imperative for making the correct diagnosis of this haemolytic anaemia. Patients with PKD are at risk for a range of complications, regardless of the degree of their anaemia. Therefore, diagnosis is key for appropriate monitoring and management. With the potential for targeted treatment, diagnosing patients with a specific type of haemolytic anaemia is even more critical so that these therapies can be considered by patients early in their disease course to potentially improve their symptoms and modify their risk of future complications.

## Acknowledgements

We appreciate the feedback and critical review of this manuscript from Dr. Bertil Glader and Professor Alberto Zanella.

## Author contributions

WB, ML, and RFG reviewed the literature and wrote the manuscript.

## Disclosures

WB, ML, and RFG are scientific advisors to Agios Pharmaceuticals. RFG receives research funding from Agios Pharmaceuticals.

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