**Diagnosis and Management of Pyruvate Kinase Deficiency: International Expert Guidelines**

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**SUMMARY**

Pyruvate kinase deficiency (PK deficiency) is the most common cause of chronic congenital non-spherocytic hemolytic anemia worldwide, with an estimated prevalence of 1 in 100,000 to 1 in 300,000 persons. PK deficiency results in chronic hemolytic anemia, with wide-ranging and serious consequences impacting health, quality of life, and mortality. The goal of the International Guidelines for the Diagnosis and Management of Pyruvate Kinase Deficiency was to develop evidence-based guidelines for the clinical care of patients with PK deficiency. This clinical guideline, funded by an unrestricted grant, was developed using GRADE methodology and the AGREE II framework. Experts were invited after consideration of area of expertise, scholarly contributions in PK deficiency, and country of practice for global representation. The expert panel included 29 expert physicians (including adult and pediatric hematologists and other subspecialists), geneticists, laboratory specialists, nurses, a guidelines methodologist, patients with PK deficiency, and caregivers from 10 countries. Five key topic areas were identified, the panel prioritized key questions, and a systematic literature search was performed to generate evidence summaries, which were used in development of draft recommendations. The expert panel then met for a guidelines conference to finalize and vote on recommendations according to a structured consensus procedure. Agreement of ≥67% among the expert panel was required for inclusion of a recommendation in the final guideline. The expert panel agreed on 31 total recommendations across five key topic areas: Diagnosis and Genetics of Pyruvate Kinase Deficiency, Monitoring and Management of Chronic Complications in Pyruvate Kinase Deficiency, Standard Management of Anemia in Pyruvate Kinase Deficiency, Targeted and Advanced Therapies in Pyruvate Kinase Deficiency, and Special Populations in Pyruvate Kinase Deficiency. These new guidelines should facilitate best practices and evidence-based PK deficiency care into clinical practice.

**SEARCH STRATEGY AND SELECTION CRITERIA**

Evidence for this guideline was systematically identified and evaluated utilizing five search strategies in Ovid MEDLINE and Ovid Embase, described in full detail in the Appendix, pp.4-14. The main searches were conducted on May 8, 2023. Two reviewers reviewed the titles and abstracts of each record and independently applied the inclusion criteria to all search results to identify full text articles to be retrieved for further review. Both reviewers then independently reviewed the full text and indicated whether each study met the inclusion criteria. Where both reviewers agreed, the study was included and progressed to the data extraction stage. Any disagreements were reviewed and resolved by discussion until concordance was fully satisfied. Included references were then compiled into evidence summaries, which were then utilized by the Guidelines Working Group throughout the development of guideline recommendations.

**INTRODUCTION**

Pyruvate kinase deficiency (PK deficiency) is the most common cause of chronic congenital non-spherocytic hemolytic anemia worldwide, affecting at least 1 in 100,000 to 1 in 300,000 individuals.1-3 An autosomal recessive disorder, PK deficiency results from mutations in the *PKLR* gene that encodes erythrocyte pyruvate kinase, an enzyme critical for erythrocyte energy production and therefore normal erythrocyte function and lifespan.4 PK deficiency is characterized by chronic hemolytic anemia of variable severity, ranging from a mild asymptomatic anemia to a life-threatening transfusion-dependent anemia, as well as many chronic complications of hemolysis, including iron overload, reduced bone density, and cardiopulmonary complications.3,5,6 PK deficiency is associated with increased overall mortality,7,8 underscoring the significance of this diagnosis and its proper management. The clinical manifestations of PK deficiency also result in impairment of health-related quality of life (HRQoL),9-11 which can be improved with effective disease management.12-14 Accurate and timely diagnosis of PK deficiency is critical for the administration of proper treatments, the diagnosis, monitoring, and prevention of disease complications, family planning and pregnancy care, and appropriate general medical care.3,15

 The goal of the International Guidelines for the Diagnosis and Management of Pyruvate Kinase Deficiency (hereafter referred to as the “International PKD Guidelines”) was to develop comprehensive clinical guidelines, informed by the best available evidence, to improve the care of patients with PK deficiency worldwide. As it is a rare disorder, the existence of such guidelines is critical for universal access to expert and evidence-based diagnostic and care recommendations. In consideration of resource limitations in low- and middle-income countries, where possible, these guidelines include potential alternative management strategies if the recommended strategy is not possible.

**METHODS**

 Recommendations for the International PKD Guidelines were developed using the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) methodology16 and conformed with the Appraisal of Guidelines for Research and Evaluation II (AGREE II) framework17 and with a guideline methodologist (N.S.). Five key topic areas were defined based on input of PK deficiency experts and the PK deficiency patient community. To maximize quality, generalizability, and applicability, the process was systematic and evidence-based, with incorporation of expert opinion where evidence was lacking, using a transparent and structured consensus procedure.

 The Guidelines Working Group (GWG, expert panel), led by co-chairs (H.A-S. and R.F.G.), included clinical and genetic experts in PK deficiency from 10 countries including multiple medical specialties and subspecialties, a guidelines methodologist, laboratory specialists, health care workers, patient advocacy representatives, caregivers of children with PK deficiency, and adults with PK deficiency (**Appendix**, p.3). Patient representatives were included at every step of the guideline development process. GWG members were assigned to one key topic group according to their experience and expertise and developed key questions and prioritized outcomes to guide a formal literature review and ultimate development of recommendations. The formal systematic literature search and development of evidence summaries, led by a medical librarian (K.L-R.) with input from the GWG co-chairs, was completed between April and June 2023. Through a prespecified literature review process (**Appendix**, pp.4-14), including review of each citation in duplicate, 368 articles were retrieved in full text for further review and those meeting inclusion criteria were summarized in evidence summaries. Prior to the consensus conference, key topic groups developed draft recommendations utilizing GRADE16. Recommendation strength was categorized as strong or conditional and further defined in the **Appendix**, p.15.Draft recommendations were distributed to all GWG members prior to the consensus conference (which took place in July 2023 in Boston, Massachusetts, USA). All GWG members completed disclosures that were reviewed by the GWG co-chairs and distributed to the GWG prior to the conference.

 At the conference, a structured consensus procedure was strictly followed (**Appendix**, p.15) following presentation of evidence summaries and recommendations. Anonymous voting was used with a prespecified agreement of ≥67% for inclusion in the final guidelines. GWG members abstained from voting in the setting of significant conflict of interest (e.g., first or senior authorship on clinical trial publications considered key evidence in the drafting of the recommendation). Recommendations failing to reach this agreement threshold on initial vote underwent review under a structured process to identify areas of disagreement, followed by revision and a second vote, also requiring ≥67% agreement for inclusion of the revised recommendation. The draft manuscript and recommendations underwent comprehensive external review by 22 topic experts, general hematologists, and guidelines experts who were not involved with the guideline creation, and their comments were collected and addressed (**Appendix,** pp.41-42). The funding source did not participate in the guideline development process at any stage, including the selection of the GWG; the design, reporting, or implementation of the guidelines; the manuscript and its review process; or the decision to submit the guidelines for publication. No representative from the funding source was invited or present at any guidelines planning meeting or the consensus conference.

**RECOMMENDATIONS**

The **Table** summarizes the guideline recommendations. Comprehensive clinical considerations and a detailed discussion and citations of references that formed the basis for each recommendation are included in full in the **Appendix** (pp.16-38). Draft recommendations not achieving the agreement threshold are included in the **Appendix** (p.43).

1. **Diagnosis and Genetics of Pyruvate Kinase Deficiency**

The clinical presentation of PK deficiency includes the usual hallmarks of chronic hemolysis—anemia, jaundice, abnormalities in laboratory hemolysis markers—that are also seen in other forms of hereditary hemolytic anemia.3,5,6 The diagnosis may be made from the newborn period to adulthood due to variability in the degree of hemolysis and disease manifestations. The rarity of the disease and wide clinical spectrum may result in diagnostic challenges, including misdiagnosis and underdiagnosis.18 Laboratory diagnosis of PK deficiency ultimately depends upon the demonstration of decreased enzyme activity and the identification of causative mutations in *PKLR* gene.18

*Recommendation A1: The expert panel recommends testing for pyruvate kinase deficiency in all patients with non-immune hemolytic anemia after exclusion of hemoglobin disorders and erythrocyte membrane disorders.*(Certainty of evidence: moderate; strength of recommendation: strong; agreement, 91%).

*Evidence/clinical considerations:* Given the phenotypic variability, misdiagnosis of patients with PK deficiency is possible.18,19 PK deficiency should be considered in all patients after both hemoglobinopathies and membranopathies have been excluded, particularly in those with evidence of dyserythropoiesis (including those originally diagnosed with a congenital dyserythropoietic anemia but not confirmed at the molecular level),20 all patients with unexplained compensated or transfusion-dependent anemia, and neonates with unexplained indirect hyperbilirubinemia. Given that inherited red cell abnormalities are very common, co-inheritance with other erythrocyte defects should be considered.

*Recommendation A2. The expert panel recommends initial testing for pyruvate kinase deficiency using either* PKLR *gene molecular analysis or pyruvate kinase enzyme activity (when performed following established testing guidance) as both methods currently have similar performance in the diagnosis of pyruvate kinase deficiency.* (Certainty of evidence: moderate; strength of recommendation: strong; agreement, 95%).

*Evidence/clinical considerations:* Where possible, the diagnostic evaluation for PK deficiency should be performed in an expert reference center. The sensitivity of PK enzyme testing (~80-90%) may be improved (>95%) when evaluated against another age-dependent red cell enzyme (PK:hexokinase ratio).21,22 The choice to use molecular testing or enzyme assay as the first step in diagnosis is directed by clinical circumstances (for example, PK enzyme assays are unreliable if a red cell transfusion has been administered in the preceding 90 days),18 by test availability, and payor/national health system recommendations and coverage. Over 300 known pathogenic mutations in *PKLR* have been identified.4 Single-gene *PKLR* exon sequencing as well as multi-gene next-generation sequencing (NGS) hemolytic anemia panels are both reliable methods for PK deficiency diagnosis23,24 but may result in false negative testing due to pathogenic intronic variants, mutations in regulatory regions, non-canonical splice site mutations, or large deletions.19,24

*Recommendation A3. The expert panel recommends confirmation of a diagnosis of pyruvate kinase deficiency initially made with pyruvate kinase enzyme activity measurements with PKLR gene molecular analysis.* (Certainty of evidence: moderate; strength of recommendation: strong; agreement, 91%).

*Evidence/clinical considerations:* Upon identification of decreased PK enzyme activity, *PKLR* genotyping should be performed where possible as enzyme activity alone cannot reliably discriminate between the homozygote/compound heterozygote (disease) state and the heterozygote (carrier) state, cannot distinguish primary (congenital) from secondary (acquired) PK deficiency, and may be reduced due to mutations in genes other than *PKLR* (e.g. *KLF1* or *GATA1*).18,25,26

*Recommendation A4.**The expert panel recommends confirmation of a diagnosis of pyruvate kinase deficiency initially made with PKLR gene molecular analysis with pyruvate kinase enzyme activity measurement in patients without two known pathogenic mutations in PKLR.* (Certainty of evidence: moderate; strength of recommendation: strong; agreement, 100%).

*Evidence/clinical considerations:* Confirmatory reduced PK enzyme activity should be obtained where possible to confirm pathogenicity of novel *PKLR* variants or variants of unknown significance detected by molecular testing.18,19

*Recommendation A5. The expert panel recommends against the use of pyruvate kinase enzyme activity predicting disease severity or disease course.* (Certainty of evidence: moderate; strength of recommendation: strong; agreement, 96%).

*Evidence/clinical considerations:* No relationship exists between PK enzyme activity and either genotype or clinical phenotype.18,19 Therefore, the utility of PK enzyme testing is limited to initial diagnostic testing only.

1. **Monitoring and Management of Chronic Complications of Pyruvate Kinase Deficiency**

PK deficiency may result in a wide range of chronic complications, including iron overload, bone mineral density disorder, cardiopulmonary disease, and others.3,5,6 Comprehensive longitudinal data related to iron overload are not available in PK deficiency. However, the PK Deficiency Natural History Study provided evidence that iron overload develops even in the absence of red cell transfusions,6,27 as is observed in other chronic hemolytic anemias such as thalassemia. There are also data which suggest that individuals with PKD have ineffective erythropoiesis, based on hepcidin, erythroferrone and other markers.20 Therefore, as in thalassemia, dysregulated iron metabolism and increased iron absorption from the intestine is believed to account for the development of iron overload in the absence of transfusions. Given this, the expert panel both utilized available data for PK deficiency and extrapolated from the extensive data available in thalassemia to inform development of these recommendations. Tissue iron and serum ferritin thresholds were derived from the thalassemia literature and used to determine optimal goals for monitoring and treatment in PK deficiency.

*Recommendation B1. The expert panel recommends screening for iron overload with serum ferritin in children and adults with pyruvate kinase deficiency to detect and avoid complications of iron overload, irrespective of transfusion status, beginning at 3 years of age or after 12 transfusion episodes, whichever occurs first.* (Certainty of evidence: low; strength of recommendation: strong; agreement, 100%).

*Evidence/clinical considerations:* While evidence highlighting the risk, consequences, and management of iron overload in PK deficiency is limited,6,27 existing data are concordant with more exhaustive studies undertaken in patients with non-transfusion dependent thalassemia and transfusion-dependent thalassemia.28,29 This concordance underscores the robustness of the recommendations. Iron overload results in several complications, all of which can potentially be avoided with effective iron chelation therapy.28,29 Hence early assessment and initiation of chelation when appropriate could prevent complications. Ferritin-based monitoring frequency should be customized to each patient, although it must be conducted at least once a year. For patients receiving regular red cell transfusions, ferritin should be assessed every 1 to 3 months. Given that ferritin is an acute phase reactant, elevated levels in the context of inflammation should be cautiously interpreted, with a repeat assessment advised before proceeding with MRI. For example, it is unlikely that a child who has not received transfusions would develop iron overload by the age of 3 years regardless of the ferritin level.

*Recommendation B2*. *The expert panel recommends measurement of liver iron concentration using magnetic resonance imaging (MRI) in children and adults with pyruvate kinase deficiency with consistent serum ferritin measurements >500 ng/mL to detect and avoid complications of hepatic iron overload, irrespective of transfusion status.* (Certainty of evidence: low; strength of recommendation: strong; agreement, 100%).

*Evidence/clinical considerations:* Liver iron overload may result in complications including cirrhosis and hepatocellular carcinoma, with significant morbidity and mortality.30 Where possible, MRI assessment of liver iron concentration (LIC) is strongly recommended. For patients receiving regular red cell transfusions or undergoing iron chelation therapy, annual liver T2, T2\*, R231 or R2\* MRI32 should be performed. Patients who are not regularly transfused may have LIC measurements less frequently, based on trends in serum ferritin, but at least once every 5 years. For non-transfused young children, the initial liver MRI may be deferred until after age 5 years to balance the risks of sedation with the utility of the information to be gained from the MRI. It is unlikely that a child not receiving transfusions would develop iron overload by the age of 3-5 years. If the serum ferritin is consistently elevated at a young age, other causes (infection, inflammation) should be considered before an MRI with sedation is performed.

*Recommendation B3. The expert panel recommends cardiac iron measurement using magnetic resonance imaging in all patients with pyruvate kinase deficiency with liver iron concentration greater than 7 mg/g dry weight to detect and avoid complications of cardiac iron overload, irrespective of transfusion status.* (Certainty of evidence: low; strength of recommendation: strong; agreement, 92%).

*Evidence/clinical considerations:* Cardiac iron overload may result in complications including heart failure and arrhythmias, with significant morbidity and mortality.33 Where possible, cardiac T2\* MRI assessment of myocardial iron may identify patients at risk for these complications and guide chelation therapy. Generally, experts agree that the risk of cardiac iron overload increases if the LIC exceeds 7 mg/g dry weight. In all patients with an LIC above 7 mg/g dry weight and in patients with a lower LIC, but who likely had periods with LIC greater than 7 mg/g dry weight in the past, cardiac iron should be assessed using MRI. The cardiac T2\* should be maintained above 20 milliseconds which correlates with minimal or no iron deposition.34 Cardiac T2\* and LIC do not correlate well since cardiac loading and unloading are both slower than in the liver.30 Children should have their first cardiac T2\* at age 10 years if they are not regularly transfused or are transfused but well chelated. In regularly transfused patients, monitoring is recommended every year (if high LIC, or ineffective chelation) or 2 years (if LIC in target range and effectively chelated). In patients not on regular transfusions, cardiac T2\* may be done less frequently depending on the LIC.

*Recommendation B4. The expert panel recommends iron chelation therapy in patients with pyruvate kinase deficiency aged 2 years or older who have a liver iron concentration exceeding 5 mg/g dry weight, irrespective of transfusion status, to reduce the risk of complications from iron overload.* (Certainty of evidence: low; strength of recommendation: strong; agreement, 100%).

*Evidence/clinical considerations:* Chelator agent selection, dosing, and monitoring is described in detail elsewhere.35 Where possible, continued MRI LIC monitoring is important to determine the effectiveness of chelation and tailoring of the regimen. In individuals not receiving regular transfusions who have slower iron loading, LIC monitoring is important to determine discontinuation of chelation so as not to over-chelate or cause chelator toxicity (possible when LIC is <2 mg/g dry weight).35 In regularly transfused individuals with continued more rapid loading, annual LIC assessment will guide tailoring of the chelation regimen.

*Recommendation B5. The expert panel recommends iron chelation therapy in patients with pyruvate kinase deficiency aged 2 years or older who have received >12 transfusions, or serum ferritin >1000 ng/ml, to reduce the risk of complications from iron overload.* (Certainty of evidence: low; strength of recommendation: strong; agreement, 100%).

*Evidence/clinical considerations:* Where LIC is not available, serum ferritin and/or red cell transfusion thresholds can be used to trigger initiation of iron chelation.36 Continued serum ferritin monitoring is important in these patients to determine chelation effectiveness and tailor the regimen.

*Recommendation B6. The expert panel suggests echocardiography in all patients with pyruvate kinase deficiency 18 years of age or older to screen for pulmonary hypertension.* (Certainty of evidence: very low; strength of recommendation: conditional; agreement, 100%).

*Evidence/clinical considerations:* Pulmonary hypertension (PH) is an uncommon complication in PK deficiency,6 associated with significant morbidity with severe impact on HRQoL.9 Screening with echocardiography utilizing the tricuspid regurgitation jet method may promote early detection of PH and thus timely intervention,37 thereby potentially improving patient outcomes. The frequency of screenings should range between 1 and 5 years and should be tailored based on individual risk factors such as relevant symptoms, prior echocardiographic measurements, and history of splenectomy.

*Recommendation* B7. *The expert panel recommends annual 25-hydroxy vitamin D measurement beginning at one year of age in all patients with pyruvate kinase deficiency not on regular vitamin D supplementation to detect and treat vitamin D deficiency and reduce the risk of bone density loss.* (Certainty of evidence: low; strength of recommendation: strong; agreement, 100%).

*Evidence/clinical considerations:* Early-onset reduced bone mineral density, resulting in heightened fracture risk, is common in patients with PK deficiency irrespective of their transfusion status,6,38 underscoring the importance of screening and treating all patients for vitamin D deficiency.

*Recommendation B8. The expert panel recommends screening for reduced bone mineral density using dual-energy x-ray absorptiometry (DEXA) scanning in all patients with pyruvate kinase deficiency beginning at 18 years of age to diagnose and manage low bone mineral density, osteopenia, and osteoporosis.* (Certainty of evidence: low; strength of recommendation: strong; agreement, 92%).

*Evidence/clinical considerations:* The frequency of monitoring should be tailored according to the results of prior DEXA scans and individual risk factors, such as fracture history, vitamin D status, and low levels of physical activity. Antiresorptive and osteoanabolic therapies can be initiated when appropriate to maintain and improve bone density. Young patients diagnosed with low bone density (Z-score ≤ −2.0 in women of childbearing potential and men <50 years of age) and older patients diagnosed with osteoporosis (T-score ≤2.5 in women of nonchildbearing potential and men ≥50 years of age) should be referred to an endocrinologist where possible to manage the treatment and oversee further DEXA monitoring.

*Recommendation B9*. *The expert panel recommends age-appropriate laboratory endocrine monitoring in patients with pyruvate kinase deficiency receiving regular transfusions and non-transfused patients who have iron overload, defined as serum ferritin >1000 ng/mL or liver iron concentration >5 mg/g dry weight, to identify and treat endocrinologic complications of iron overload.* (Certainty of evidence: very low; strength of recommendation: strong; agreement, 100%).

*Evidence/clinical considerations:* The heightened risk of thyroid, pancreatic, or pituitary dysfunction in patients with PK deficiency primarily pertains to those with iron overload.6,27,30 Therefore, enhanced screening can be limited to these individuals and directed by an endocrinologist. Because hemoglobin A1c measurements are unreliable in hemolytic anemia,39 annual diabetes screenings utilizing alternative measures (fasting glucose, oral glucose tolerance testing, or fructosamine measurement) are recommended.

*Recommendation B10. The expert panel suggests monitoring of renal function in children and adults with pyruvate kinase deficiency, irrespective of transfusion status for early detection of renal dysfunction.* Certainty of evidence: low; strength of recommendation: conditional; agreement, 68%).

*Evidence/clinical considerations:* Kidney disease including hyperfiltration, hypercalciuria and albuminuria in hemolytic anemias is believed to occur from chronic anemia, ongoing hemolysis, and free heme-mediated renal damage, as well as iron-mediated glomerular injury. Hemolysis-related renal injury can result from reactive oxygen species production and activation of inflammatory pathways.40 Iron chelators, such as deferasirox, may additionally lead to renal tubular abnormalities including renal tubular acidosis.41 Based on the available data for other congenital hemolytic anemias, the expert panel suggests monitoring renal parameters including creatinine, phosphorus, magnesium, albuminuria and urine protein:creatinine ratio based on individual risk factors (underlying kidney dysfunction, chelation drugs).

1. **Standard Management of Anemia in Pyruvate Kinase Deficiency**

Splenectomy and red cell transfusion are the two standard supportive treatment approaches to managing anemia and associated symptoms in PK deficiency. As for most rare diseases, there are no randomized controlled trials (RCTs) defining how or when these interventions should be used, and the recommendations in this section are based on observational and registry studies. A brief discussion of folic acid use in PK deficiency can be found in the **Appendix**, pp.26-27.

Because of the lack of evidence, it was not possible to make recommendations regarding some important aspects of treatment, particularly whether regular transfusions or splenectomy should be used as the first line treatment for patients with chronic symptoms of anemia. This management decision-making has become complicated in adults with the emergence of PK activators and will become increasingly complex as more effective drugs emerge and trials are completed in children.

*Recommendation C1*. *The expert panel recommends discussion of the individualized risks and benefits of splenectomy to treat anemia in children greater than 5 years old and adults who require regular or frequent red cell transfusions or who have symptomatic anemia, to reduce transfusion burden and alleviate symptoms.* (Certainty of evidence: moderate; strength of recommendation: strong; agreement, 96%).

*Evidence/clinical considerations:* In a large observational study including 150 patients with PK deficiency who underwent splenectomy, the transfusion burden was reduced in 90% and hemoglobin increased by a mean of 1.6 g/dL, although 20% had no response to splenectomy.6 Patients with higher pre-splenectomy hemoglobin, lower bilirubin and missense *PKLR* variants were more likely to respond. Splenectomy is not curative and potential benefits must be balanced against the risks, including life-threatening infection,42,43 thromboembolic disease, and late cardiovascular complications, including pulmonary hypertension and ischemic heart disease.44 Given this, the expert panel recommends that risks and benefits are discussed with families, adults and children with PK deficiency, and decisions made based on individual preferences, availability of safe transfusions and iron chelation, and access to newer therapies. Additional recommendations regarding vaccination and post-splenectomy antibiotic prophylaxis are given in the Appendix, pp.25-26.

*Recommendation C2. The expert panel recommends initiation of regular red cell transfusions in children <5 years of age with pyruvate kinase deficiency who have symptomatic anemia or anemia that has an impact on growth and development, to improve anemia symptoms and growth.* (Certainty of evidence: low; strength of recommendation: strong; agreement, 91%).

*Evidence/clinical considerations:* Due to a lack of evidence in PK deficiency, evidence is inferred from studies and guidelines on the management of other transfusion-dependent conditions such as thalassemia. Regular transfusion typically involves giving regular transfusions every 3-12 weeks to optimize childhood growth and development and treat symptoms of anemia.

*Recommendation C3.* *The expert panel recommends treatment with regular red cell transfusions in children 5 years of age and older and adults with pyruvate kinase deficiency who have symptomatic anemia despite splenectomy or are unsuitable for or unwilling to undergo splenectomy to improve symptoms of anemia.* (Certainty of evidence: low; strength of recommendation: strong; agreement, 92%).

*Evidence/clinical considerations*: Compared with splenectomy, regular transfusion offers a more predictable increase in hemoglobin and can be modified or discontinued as circumstances change but requires much more time in hospitals and long-term iron chelation. Additional risks of regular transfusion include blood-borne infections, red cell alloimmunization, and the need for regular intravenous access. Optimization of childhood growth and development is critical; in adults, regular transfusion is administered primarily to alleviate anemia symptoms and improve HRQoL and the decision to initiate them is individualized between provider and patient.

*Recommendation C4. The expert panel recommends that red cell transfusions be administered to children and adults with pyruvate kinase deficiency on the basis of anemia symptoms and complications rather than a universal hemoglobin transfusion threshold.* (Certainty of evidence: low; strength of recommendation: strong; agreement, 87%).

*Evidence/clinical considerations:* In PK deficiency, tolerance of a certain degree of anemia varies widely between individuals and within the same individual at different points in the lifespan due to various factors. The decision to administer unplanned/episodic transfusions should be based on a combination of symptoms, laboratory results, circumstances, and patient/family preference, not on falling below or remaining above an arbitrary hemoglobin threshold.

*Recommendation C5. The expert panel suggests that if a splenectomy is planned in a patient with pyruvate kinase deficiency, cholecystectomy is also considered and discussed with the patient, family, and/or caregiver.* (Certainty of evidence: low; strength of recommendation: conditional; agreement, 96%).

*Evidence/clinical considerations*: Gallstone disease is common in PK deficiency as a complication of chronic hemolysis.6 Given this risk, cholecystectomy at the time of a splenectomy may be beneficial and reduce the risk of future biliary complications, especially in patients with known gallstones or biliary sludge.

*Recommendation C6. The expert panel recommends that appropriate psychological support be offered to children and adults with pyruvate kinase deficiency and their families and/or caregivers.* (Certainty of evidence: low; strength of recommendation: strong; agreement, 100%).

*Evidence/clinical considerations:* As with many chronic medical conditions, comorbid psychiatric complications including anxiety and depression are common in patients with PK deficiency and their caregivers.45 Therefore, mental health should be discussed with patients and caregivers and services should be made available where appropriate.

1. **Targeted and Advanced Therapies in Pyruvate Kinase Deficiency**

The advent of a targeted activator of pyruvate kinase, mitapivat, has ushered in a new era of advanced therapy for patients with PK deficiency responsive to this treatment.14 The only placebo-controlled RCT, ACTIVATE,12 was performed in adult patients not receiving regular transfusions, while a careful prospective single-arm trial, ACTIVATE-T,13 was conducted in adults receiving regular transfusions.

Clinical trials of mitapivat in non-transfused46 and transfused47 pediatric patients are ongoing. Allogeneic transplantation is considered by members of the expert panel to be of potential use in carefully selected patients, but the published outcomes in case series have not been promising,48 and few conclusions can be reached. Novel approaches, including gene therapy, are currently under study and are premature for recommendations from the expert panel at this time.

*Recommendation D1. The expert panel recommends initiation of mitapivat therapy in adult patients with pyruvate kinase deficiency who are anemic, who are not regularly transfused, and who do not have two non-missense mutations, irrespective of splenectomy status, to improve hemoglobin and health-related quality of life.* (Certainty of evidence: high; strength of recommendation: strong; agreement, 100%).

*Evidence/clinical considerations:* This recommendation is based on the ACTIVATE phase 3 RCT for mitapivat.12 The qualifications for age, transfusion status, and mutation status in the recommendation were eligibility criteria for enrollment on the ACTIVATE trial. The observation that mitapivat could be effective, regardless of splenectomy status, was made in both the phase 2 and 3 studies. Because mitapivat is an allosteric activator of the PK enzyme, it lacks activity if the protein is absent (e.g. in the presence of two null mutations, **Supplementary Table**).49 In addition to improvement of anemia manifestations and HRQoL, data are emerging suggesting that mitapivat may ameliorate chronic complications of hemolysis, notably iron overload.50 Along with monitoring for potential adverse effects of treatment, standard monitoring for complications of PK deficiency should be continued in patients receiving mitapivat.

*Recommendation D2. The expert panel recommends that “failure to respond” to mitapivat in patients with pyruvate kinase deficiency who are not regularly transfused should be declared only after at least 3 months of treatment with mitapivat at an optimal or maximum dose.* (Certainty of evidence: high; strength of recommendation: strong; agreement, 100%).

*Evidence/clinical considerations:* The expert panel recommends dose optimization of mitapivat according to the drug prescribing information. For many subjects in the mitapivat arm of the ACTIVATE trial,12 hemoglobin improvement occurred less than a month after initiation at an optimal dose (**Supplementary Figure**). However, as potential delay in observing a benefit might arise (for example, due to an intercurrent illness), three months should be sufficient to assess hemoglobin response in most patients.

*Recommendation D3. The expert panel recommends initiation of mitapivat therapy in adult patients with pyruvate kinase deficiency who are regularly transfused and who do not have two non-missense mutations, irrespective of splenectomy status, to reduce transfusion burden.* (Certainty of evidence: moderate; strength of recommendation: strong; agreement, 92%).

*Evidence/clinical considerations:* This recommendation is based directly upon results of the prospective, single-arm phase 3 ACTIVATE-T trial of adults with PK deficiency receiving regular transfusions.13 This trial demonstrated ≥33% reduction in transfusion burden in 37% of patients, including 22% who achieved complete transfusion independence.

*Recommendation D4. The expert panel recommends discontinuation of mitapivat therapy, and return to best supportive care, in patients with pyruvate kinase deficiency who are non-responders to mitapivat, irrespective of transfusion status. (Certainty of evidence: moderate; strength of recommendation: strong; agreement, 92%).*

*Evidence/clinical considerations:* Neither ACTIVATE nor ACTIVATE-T directly address management strategies in mitapivat non-responders as a trial outcome, but all of the alternative treatment strategies discussed herein are available for mitapivat non-responders. The expert panel acknowledges the fact that both splenectomy status and transfusion status are related to patient/physician decision making, not necessarily to underlying biology. Therefore, in a mitapivat non-responding patient, splenectomy may be a subsequent option, and if the patient was not previously transfused, a strategy of chronic transfusions plus iron chelation is an option.

*Recommendation D5. The expert panel recommends consideration of alternative approaches, including clinical trials, in patients with pyruvate kinase deficiency who are non-responders to mitapivat, irrespective of transfusion status. (Certainty of evidence: very low; strength of recommendation: strong; agreement, 100%).*

*Evidence/clinical considerations:* Despite a paucity of high-quality evidence, this recommendation is included by the panel to acknowledge the importance of novel therapeutic approaches in non-responders to mitapivat. Trials of alternative approaches are now available or may become available in the future. A case series of 16 patients with PK deficiency receiving allogenic hematopoietic stem cell transplant has been published.48 Lentiviral-mediated gene therapy has been studied in a phase 1 trial of patients with PK deficiency,51 but the data are too preliminary for an expert panel recommendation at this time.

*Recommendation D6.* *The expert panel recommends discontinuation of mitapivat therapy in patients with pyruvate kinase deficiency receiving regular transfusions who do not achieve at least a 33% reduction in transfusion requirement, with the exception of patients who achieve marked improvement in iron status, patient-reported health outcomes, jaundice, or other key disease parameters.* (Certainty of evidence: low; strength of recommendation: strong; agreement, 88%).

*Evidence/clinical considerations:* In the ACTIVATE-T trial, the proportion of patients with a transfusion reduction of ≥33% was 37% over a duration of 24 weeks.13 The review panel found it plausible that outcomes other than transfusion reduction success might be clinically meaningful and merit continued mitapivat treatment. This includes the ability to be more effectively chelated,50 improved patient-reported outcome (PRO) measures, or significantly improved jaundice. The trial was not designed for these outcomes to be declared “successes,” but they might be reasonable cause to continue the medication in some selected transfused patients, with the caveat that mitapivat should not be considered as only an adjunctive iron chelator without other evidence of improvements.

*Recommendation D7.* *The expert panel recommends that adults with pyruvate kinase deficiency who are regularly transfused and who have not undergone splenectomy receive a trial of mitapivat therapy prior to consideration of splenectomy.* (Certainty of evidence: low; strength of recommendation: strong; agreement, 100%).

*Evidence/clinical considerations: S*plenectomy is irreversible and comes with potential irreversible harms and heightened lifelong risks,6,52 while mitapivat can be easily discontinued and has demonstrated a favorable safety profile in clinical trials.12,13,49

*Recommendation D8.* *The expert panel suggests that pyruvate kinase deficiency-specific measures of health-related quality of life (patient-reported outcomes) can be a determining factor of success in an individual trial of mitapivat in cases where the reduction in transfusion burden or increase in hemoglobin falls short of an arbitrary numerical cutoff.* (Certainty of evidence: moderate; strength of recommendation: conditional; agreement, 82%).

*Evidence/clinical considerations:* In both ACTIVATE and ACTIVATE-T, responders had improvement in disease-specific PRO measures as secondary endpoints.12,13 The expert panel recognizes that PRO measures are therefore important in judging success or failure of treatment.11,53 Taking PRO measures into account may be an ideal demonstration of value to heath authorities and payers when therapeutic response falls short of a particular hemoglobin increment or transfusion reduction target.

1. **Special Populations in Pyruvate Kinase Deficiency**

Distinct clinical monitoring and treatment needs have been identified at different ages and during pregnancy, which are critical to address with specific testing and management recommendations.

*Recommendation E1. The expert panel recommends regular monitoring of children and adults with pyruvate kinase deficiency by a hematologist, irrespective of transfusion status.* (Good Practice statement; agreement, 96%)

*Evidence/clinical considerations:* For infants diagnosed at birth, where possible, the expert panel recommends hematologist monitoring in close partnership with the general pediatrician to manage hyperbilirubinemia and growth at 2, 4 and 12 weeks of age, followed by a transition to monitoring visits every 3 months (with interval primary care visits at 2 and 4 months of age). Children under 5 years of age should be monitored by a hematologist every 3 months and children aged 5 years and older who are not receiving regular transfusions should be monitored by a hematologist every 6-12 months to evaluate for growth, physical and pubertal development, hypersplenism and signs of extramedullary hematopoiesis.54 Though they are not a special population, the expert panel felt it important to include in the guideline that adults with PK deficiency should be monitored at least annually, and usually more frequently, by a hematologist.

*Recommendation E2. The expert panel recommends that women with pyruvate kinase deficiency irrespective of transfusion status who are pregnant or are planning pregnancy be referred to a multidisciplinary feto-maternal team (including a hematologist, obstetrician, neonatologist, and other specialists as appropriate) to reduce maternal and fetal complications.* (Good Practice statement; agreement, 100%)

*Evidence/clinical considerations:* Given potential benefit to pregnancy outcomes by avoiding severe anemia, the expert panel recommends routine monitoring during pregnancy with a primary focus on anemia and its associated complications. Pregnancy is a potential trigger for increased rate of hemolysis and increased physiologic stress and may trigger institution of an intrapartum regular transfusion regimen in patients not previously receiving regular transfusions. International guidelines for thalassemia recommend maintaining maternal hemoglobin >10 g/dL to reduce maternal complications, prematurity, and fetal growth restrictions.55 In a large population based study, anemia defined as hemoglobin <10.0 g/dL was associated with an increased risk of preterm delivery, low birthweight, need for Cesarean delivery, and placental complications.56 This risk was further increased when hemoglobin was <8.0 g/dL.

**FUTURE DIRECTIONS AND DISSEMINATION AND IMPLEMENTATION STRATEGIES**

Future priorities for research and guideline development in PK deficiency as well as comprehensive dissemination and implementation strategies for the current guideline are described in detail in the **Appendix**, pp.39-40. These evidence-based guidelines will be freely and globally available to patients and clinicians, including adult and pediatric hematologists, general practitioners, other specialists, patients, and caregivers. Dissemination in multiple languages will occur via a dedicated website (pkdguidelines.org), patient brochures, online webinars, podcasts, social media, as well as endorsements and presentations from professional societies, reference networks, and patient advocacy foundations.

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**CONTRIBUTORS**

H. Al-Samkari and R. F. Grace contributed to conception and design, data collection, analysis and interpretation of the data, writing the first draft of the manuscript, critical revision of the intellectual content, final approval of the manuscript, and administrative, technical, and logistic support. K. Lang-Robertson, P. Bianchi, A. B. Glenthøj, S. Sheth, E. J. Neufeld, D. C. Rees, and S. Chonat contributed to data collection, analysis and interpretation of the data, writing the first draft of the manuscript, critical revision of the intellectual content, and final approval of the manuscript. All other authors contributed to analysis and interpretation of the data, critical revision of the intellectual content, and final approval of the manuscript.

**CONFLICT OF INTEREST STATEMENT**

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The authors certify that they do not have, within the past 3 years or with a relevant company or competitor, any stocks or shares, equity, a contract of employment, or a named position on a company board; hold (or are applying for) a relevant patent; or have been asked by anyone to write, be named on, or to submit this manuscript. Funding for this guideline, and therefore support for all authors for the present work, was provided by an unrestricted grant from Agios Pharmaceuticals. The funding source played no role in the guidelines process, including no role in the conception of the guidelines; the guideline development process at any stage; the selection of the GWG; the design, reporting, or implementation of the guidelines; the manuscript and its review process; or the decision to submit the guidelines for publication.

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**TABLE**

**Table.** Clinical recommendations from the International PK Deficiency Guidelines. COE, certainty of evidence; SOR, strength of recommendation.

|  |  |  |  |
| --- | --- | --- | --- |
| **Recommendation** | **COE** | **SOR** | **Agreement** |
| ***Diagnosis and Genetics of Pyruvate Kinase Deficiency*** |
| A1. The expert panel recommends testing for pyruvate kinase deficiency in all patients with non-immune hemolytic anemia after exclusion of hemoglobin disorders and erythrocyte membrane disorders. | Moderate | Strong | 91% |
| A2. The expert panel recommends initial testing for pyruvate kinase deficiency using either PKLR gene molecular analysis or pyruvate kinase enzyme activity (when performed following established testing guidance) as both methods currently have similar performance in the diagnosis of pyruvate kinase deficiency. | Moderate | Strong | 95% |
| A3. The expert panel recommends confirmation of a diagnosis of pyruvate kinase deficiency initially made with pyruvate kinase enzyme activity measurements with PKLR gene molecular analysis. | Moderate | Strong | 91% |
| A4. The expert panel recommends confirmation of a diagnosis of pyruvate kinase deficiency initially made with PKLR gene molecular analysis with pyruvate kinase enzyme activity measurement in patients without two known pathogenic mutations in *PKLR*. | Moderate | Strong | 100% |
| A5. The expert panel recommends against the use of pyruvate kinase enzyme activity predicting disease severity or disease course. | Moderate | Strong | 96% |
| ***Monitoring and Management of Chronic Complications of Pyruvate Kinase Deficiency*** |
| B1. The expert panel recommends screening for iron overload with serum ferritin in children and adults with pyruvate kinase deficiency to detect and avoid complications of iron overload, irrespective of transfusion status, beginning at 3 years of age or after 12 transfusion episodes, whichever occurs first.  | Low | Strong | 100% |
| B2. The expert panel recommends measurement of liver iron concentration using magnetic resonance imaging in children and adults with pyruvate kinase deficiency with consistent serum ferritin measurements >500 ng/mL to detect and avoid complications of hepatic iron overload, irrespective of transfusion status. | Low | Strong | 100% |
| B3. The expert panel recommends cardiac iron measurement using magnetic resonance imaging in all patients with pyruvate kinase deficiency with liver iron concentration greater than 7 mg/g dry weight to detect and avoid complications of cardiac iron overload, irrespective of transfusion status. | Low | Strong | 92% |
| B4. The expert panel recommends iron chelation therapy in patients with pyruvate kinase deficiency aged 2 years or older who have a liver iron concentration exceeding 5 mg/g dry weight, irrespective of transfusion status, to reduce the risk of complications from iron overload. | Low | Strong | 100% |
| B5. The expert panel recommends iron chelation therapy in patients with pyruvate kinase deficiency aged 2 years or older who have received >12 transfusions, or serum ferritin >1000 ng/ml, to reduce the risk of complications from iron overload. | Low | Strong | 100% |
| B6. The expert panel suggests echocardiography in all patients with pyruvate kinase deficiency 18 years of age or older to screen for pulmonary hypertension. | Very Low | Conditional | 100% |
| B7. The expert panel recommends annual 25-hydroxy vitamin D measurement beginning at one year of age in all patients with pyruvate kinase deficiency not on regular supplementation to detect and treat vitamin D deficiency and reduce the risk of bone density loss. | Low | Strong | 100% |
| B8. The expert panel recommends screening for reduced bone mineral density using dual-energy x-ray absorptiometry (DEXA) scanning in all patients with pyruvate kinase deficiency beginning at 18 years of age to diagnose and manage low bone mineral density, osteopenia, and osteoporosis. | Low | Strong | 92% |
| B9. The expert panel recommends age-appropriate laboratory endocrine monitoring in patients with pyruvate kinase deficiency receiving regular transfusions, and non-transfused patients who have iron overload, defined as serum ferritin >1000 ng/mL or liver iron concentration >5 mg/g dry weight, to identify and treat endocrinologic complications of iron overload.  | Low | Strong | 100% |
| B10. The expert panel suggests monitoring of renal function in children and adults with pyruvate kinase deficiency, irrespective of transfusion status for early detection of renal dysfunction. | Low | Conditional | 68% |
| ***Standard Management of Anemia in Pyruvate Kinase Deficiency*** |
| C1. The expert panel recommends discussion of the individualized risks and benefits of splenectomy to treat anemia in children greater than 5 years old and adults who require regular or frequent red cell transfusions or who have symptomatic anemia, to reduce transfusion burden and alleviate symptoms. | Moderate | Strong | 96% |
| C2. The expert panel recommends initiation of regular red cell transfusions in children <5 years of age with pyruvate kinase deficiency who have symptomatic anemia or anemia that has an impact on growth and development, to improve anemia symptoms and growth. | Low | Strong | 91% |
| C3. The expert panel recommends treatment with regular red cell transfusions in children 5 years of age and older and adults with pyruvate kinase deficiency who have symptomatic anemia despite splenectomy or are unsuitable for or unwilling to undergo splenectomy to improve symptoms of anemia. | Low | Strong | 92% |
| C4. The expert panel recommends that red cell transfusions be administered to children and adults with pyruvate kinase deficiency on the basis of anemia symptoms and complications rather than a universal hemoglobin transfusion threshold. | Low | Strong | 87% |
| C5. The expert panel suggests that if a splenectomy is planned in a patient with pyruvate kinase deficiency, cholecystectomy is also considered and discussed with the patient, family, and/or caregiver. | Low | Conditional | 96% |
| C6. The expert panel recommends that appropriate psychological support be offered to children and adults with pyruvate kinase deficiency and their families and/or caregivers. | Low | Strong | 100% |
| ***Targeted and Advanced Therapies in Pyruvate Kinase Deficiency*** |
| D1. The expert panel recommends initiation of mitapivat therapy in adult patients with pyruvate kinase deficiency who are anemic, who are not regularly transfused, and who do not have two non-missense mutations, irrespective of splenectomy status, to improve hemoglobin and health-related quality of life. | High | Strong | 100% |
| D2. The expert panel recommends that “failure to respond” to mitapivat in patients with pyruvate kinase deficiency who are not regularly transfused should be declared only after at least 3 months of treatment with mitapivat at an optimal or maximum dose. | High | Strong | 100% |
| D3. The expert panel recommends initiation of mitapivat therapy in adult patients with pyruvate kinase deficiency who are regularly transfused and who do not have two non-missense mutations, irrespective of splenectomy status, to reduce transfusion burden. | Moderate | Strong | 92% |
| D4. The expert panel recommends discontinuation of mitapivat therapy, and return to best supportive care, in patients with pyruvate kinase deficiency who are non-responders to mitapivat, irrespective of transfusion status. | Moderate | Strong | 92% |
| D5. The expert panel recommends consideration of alternative approaches, including clinical trials, in patients with pyruvate kinase deficiency who are non-responders to mitapivat, irrespective of transfusion status. | Very Low | Strong | 100% |
| D6. The expert panel recommends discontinuation of mitapivat therapy in patients with pyruvate kinase deficiency receiving regular transfusions who do not achieve at least a 33% reduction in transfusion requirement, with the exception of patients who achieve marked improvement in iron status, patient-reported health outcomes, jaundice, or other key disease parameters. | Low | Strong | 88% |
| D7. The expert panel recommends that adults with pyruvate kinase deficiency who are regularly transfused and who have not undergone splenectomy receive a trial of mitapivat therapy prior to consideration of splenectomy. | Low | Strong | 100% |
| D8. The expert panel suggests that pyruvate kinase deficiency-specific measures of health-related quality of life (patient-reported outcomes) can be a determining factor of success in an individual trial of mitapivat in cases where the reduction in transfusion burden or increase in hemoglobin falls short of an arbitrary numerical cutoff. | Moderate | Conditional | 82% |
| ***Special Populations in Pyruvate Kinase Deficiency*** |
| E1. The expert panel recommends regular monitoring of children and adults with pyruvate kinase deficiency by a hematologist, irrespective of transfusion status. | Good Practice statement | 96% |
| E2. The expert panel recommends that women with pyruvate kinase deficiency irrespective of transfusion status who are pregnant or are planning pregnancy be referred to a multidisciplinary feto-maternal team (including a hematologist, obstetrician, neonatologist, and other specialists as appropriate) to reduce maternal and fetal complications. | Good Practice statement | 100% |