Fill the gap in your knowledge, *fast!*

with ***Fast Facts*** – the ultimate medical handbook series

FAST FACTS

Pyruvate Kinase Deficiency

FAST FACTS
Pyruvate Kinase Deficiency

An independent publication developed by S. Karger Publishers Limited and provided as a service to medicine.

Supported by an educational grant from:

agios

the best offers are on
fastfacts.com

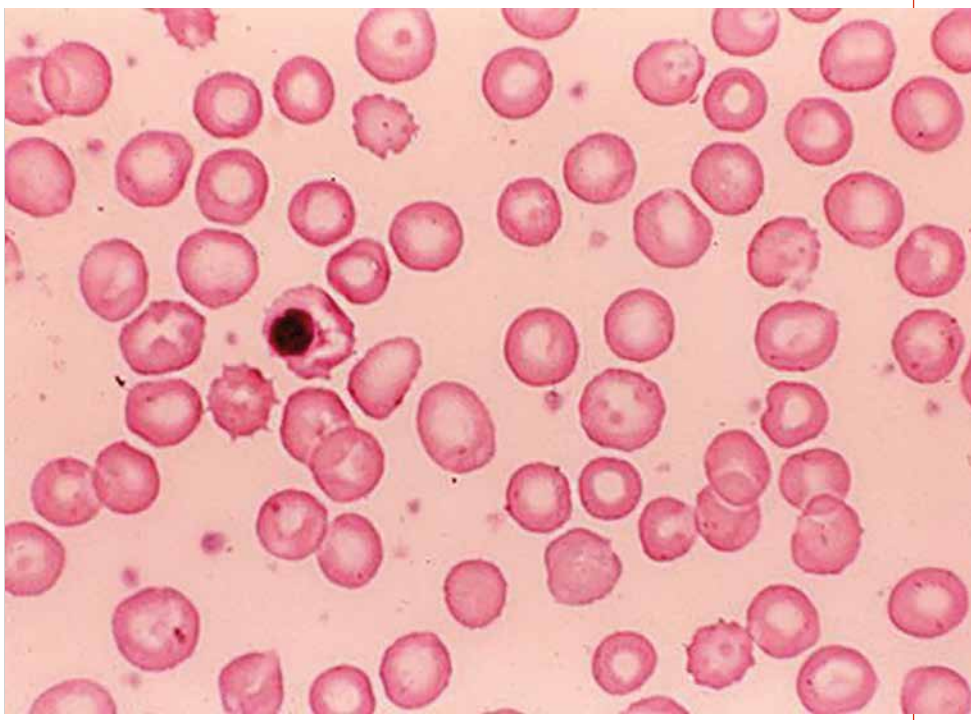
KARGER

ISBN 978-1-910797-88-4

FAST FACTS

Pyruvate Kinase Deficiency

Bertil Glader, Wilma Barcellini and Rachael Grace



Raising awareness of this rare genetic disease

FAST FACTS

Fast Facts: Pyruvate Kinase Deficiency

Bertil Glader MD PhD
Professor of Pediatrics (Hematology/Oncology)
Director, Red Blood Cell Special Studies Laboratory
Stanford University School of Medicine
Lucile Packard Children's Hospital Stanford
Palo Alto, California, USA

Wilma Barcellini MD PhD
Associate Professor in Blood Diseases, Oncology and Rheumatology, University of Milan
Director of Pathophysiology, Anemia Unit, Ospedale Maggiore Policlinico Foundation
Milan, Italy

Rachael Grace MD MMSc
Assistant Professor, Harvard Medical School
Dana-Farber/Boston Children's Cancer and Blood Disorders Center
Boston, Massachusetts, USA

Declaration of Independence
This book is as balanced and as practical as we can make it.
Ideas for improvement are always welcome: feedback@fastfacts.com

KARGER

Fast Facts: Pyruvate Kinase Deficiency
First edition June 2018

S. Karger Publishers Limited, Elizabeth House, Queen Street,
Abingdon, Oxford OX14 3LN, UK
Tel: +44 (0)1235 523233

Book orders can be placed by telephone or via the website.
To order via the website, please go to: fastfacts.com
For telephone orders, please call +44 (0)1752 202301

A CIP record for this title is available from the British Library.

ISBN 978-1-910797-88-4

Glader B (Bertil)
Fast Facts: Pyruvate Kinase Deficiency/
Bertil Glader, Wilma Barcellini, Rachael Grace

Cover photo: peripheral blood smear from a child with pyruvate kinase deficiency after splenectomy.
Medical illustrations by Graeme Chambers and Annamaria Dutto.
Typesetting by Thomas Bohm, User Design, Illustration and Typesetting, UK.
Printed in the UK with Xpedient Print.

List of abbreviations

2,3-DPG: 2,3-diphosphoglycerate

AIHA: autoimmune hemolytic anemia

ATP: adenosine triphosphate

BPGM: biphosphoglycerate mutase

CAD: cold agglutinin disease

DAT: direct antiglobulin test

DHAP: dihydroxyacetone phosphate

GAPDH: glyceraldehyde phosphate dehydrogenase

G6PD: glucose-6-phosphate dehydrogenase

G6PI: glucose-6-phosphate isomerase

GPI: glycosylphosphatidylinositol

Hb: hemoglobin

HK: hexokinase

HMP: hexose monophosphate (shunt)

Ig: immunoglobulin

LDH: lactate dehydrogenase

NAD: nicotinamide adenine dinucleotide

NADH: nicotinamide adenine dinucleotide – reduced

NADP+: nicotinamide adenine dinucleotide phosphate

NADPH: nicotinamide adenine dinucleotide phosphate – reduced

NGS: next-generation sequencing

PGK: phosphoglycerate kinase

PGM: phosphoglycerate mutase

PFK: phosphofructokinase

PK: pyruvate kinase

PNH: paroxysmal nocturnal hemoglobinuria

RBC: red blood cell

TPI: triosephosphate isomerase

Glossary

Alloantibody: an antibody produced after the introduction of an antigen into the system of an individual lacking that particular antigen

Anisocytosis: variation in the size of red blood cells

Aplastic crisis: a temporary cessation of red blood cell production, associated with infection with parvovirus B19

Cholecystectomy: surgical removal of the gallbladder

Compound heterozygote: an individual with a different mutation in each copy of a gene

Direct antiglobulin (Coombs) test: test used to detect antibodies and/or complement bound to the surface of red blood cells

Dysmyelopoiesis: impaired production of blood cells

Echinocyte: a red blood cell with an abnormal cell membrane characterized by multiple small evenly spaced projections

Erythropoiesis: production of erythrocytes

Erythropoietin: a hormone secreted by the kidneys, which stimulates red blood cell production in the bone marrow

Extramedullary hematopoiesis: the formation of blood cells in locations other than the bone marrow

Flow cytometry: an analytic cell-biology technique that utilizes light to identify, separate and characterize cells

Gilbert syndrome: a recessive condition caused by *UGT1A1* gene mutations, which results in a reduction in glucuronidation and an unconjugated hyperbilirubinemia

Glucose-6-phosphate dehydrogenase deficiency: an X-linked cell enzyme disorder, most often characterized by intermittent hemolytic episodes in the setting of oxidative stress caused by medications, infections or ingestion of fava beans

Glycolytic pathway: the 'energy-producing' metabolic pathway in red blood cells

Hematopoiesis: the formation of blood cells

Hemochromatosis: excessive accumulation of iron in the body

Hemolysis: lysis of red blood cells

Hemolytic episode: an upsurge in hemolysis, often triggered by acute infections

Hemosiderosis: excessive accumulation of iron (in the form of hemosiderin) in the body

Hepcidin: a hormone produced by the liver that reduces both the entry of iron from the gastrointestinal tract and the exit of iron from macrophages and the liver

Hexose monophosphate shunt: the 'protective' metabolic pathway that is particularly important in red blood cells as the only source of the reduced form of nicotinamide adenine dinucleotide phosphate

Hydrops fetalis: accumulation of fluid in two or more fetal compartments

Hyperbilirubinemia: excess bilirubin in the blood

Missense mutation: a single nucleotide change that results in the production of a different amino acid

Myelodysplastic syndrome: accumulation of acquired genetic changes in the bone marrow, which cause cytopenias and cell dysplasia

Myelofibrosis: replacement of the hematopoietic cells of the bone marrow by fibrosis; primary myelofibrosis is an acquired clonal myeloproliferative disorder

Next-generation sequencing: term used to describe a number of different gene sequencing technologies; also known as high-throughput sequencing

Poikilocytosis: the presence of abnormally shaped red blood cells (poikilocytes)

Polychromatophilia: excessive staining of a peripheral blood smear as a result of increased numbers of reticulocytes

Pyruvate kinase: an enzyme that catalyzes the final step in glycolysis, converting phosphoenrolpyruvate to pyruvate, which is essential for the production of red blood cell ATP (energy)

Reticulocytosis: an increase in the number of immature red blood cells (reticulocytes) in the blood, typically following hemorrhage or accompanying hemolytic anemia

Splenectomy: surgical removal of the spleen

Splenomegaly: enlargement of the spleen

Thrombocytopenia: abnormally low number of platelets

Thrombocytosis: an increase above the normal number of platelets in the blood

Thrombosis: the formation or presence of a blood clot within a blood vessel

Introduction

Red blood cell pyruvate kinase (PK) deficiency is an inherited disease manifesting as hemolytic anemia. PK deficiency is a lifelong condition, with symptoms that range from mild to severe. Despite an ever-growing understanding of its pathophysiology, etiology and epidemiology, and an active research program, PK deficiency remains unfamiliar to many medical practitioners.

Here, we provide a concise guide to PK deficiency for primary care providers, as well as hematologists, oncologists, pediatricians, internal medicine specialists, hematology nurses and medical students. As well as explaining the underlying defect, its mode of inheritance and how the condition manifests, we also discuss the diagnosis and differential diagnosis of PK deficiency, together with the complications that may arise and options for managing them.

Each chapter is supported by key learning points and references for further reading, and we encourage you to take the free online FastTest that accompanies this resource at fastfacts.com to assess your understanding of this condition.

We hope that this first edition of *Fast Facts: Pyruvate Kinase Deficiency* will be a useful resource for anyone who has an interest in learning more about this rare genetic blood disorder.

1 Overview

Pyruvate kinase (PK) deficiency is the most common enzyme deficiency affecting the glycolytic pathway used by red blood cells (RBCs) to generate energy. PK is a tetrameric protein that catalyzes the conversion of phosphoenolpyruvate to pyruvate, one of two energy-generating steps in glycolysis.

PK deficiency was first described in 1961.[1] It is inherited in an autosomal recessive manner[2] and presents as a congenital non-spherocytic hemolytic anemia.[3]

To understand the impact of PK deficiency, it is necessary to review the overall metabolism in RBCs.

Metabolic pathways in normal red blood cells

Mature RBCs lack a nucleus, ribosomes and mitochondria: as a result, they are incapable of cell division and protein synthesis, and are unable to generate energy through oxidative phosphorylation, as occurs in other cell types. Instead, RBCs rely on breaking down glucose to pyruvate and lactate to produce energy.

Two major metabolic pathways in RBCs use glucose as a substrate (Figure 1.1):

- the glycolytic, or 'energy-producing', pathway
- the hexose monophosphate (HMP) shunt (also known as the pentose phosphate pathway), or 'protective' pathway.

Glycolytic pathway. Under normal conditions, approximately 90% of glucose metabolism in RBCs occurs via the glycolytic pathway, with 5–10% metabolized via the HMP shunt.

The key products of the glycolytic pathway are:

- adenosine triphosphate (ATP), which provides energy for RBCs
- 2,3-diphosphoglycerate (2,3-DPG), an intermediate product in the pathway, which influences the release of oxygen by hemoglobin
- NADH (the reduced form of nicotinamide adenine dinucleotide), a cofactor involved in the conversion of methemoglobin to hemoglobin.

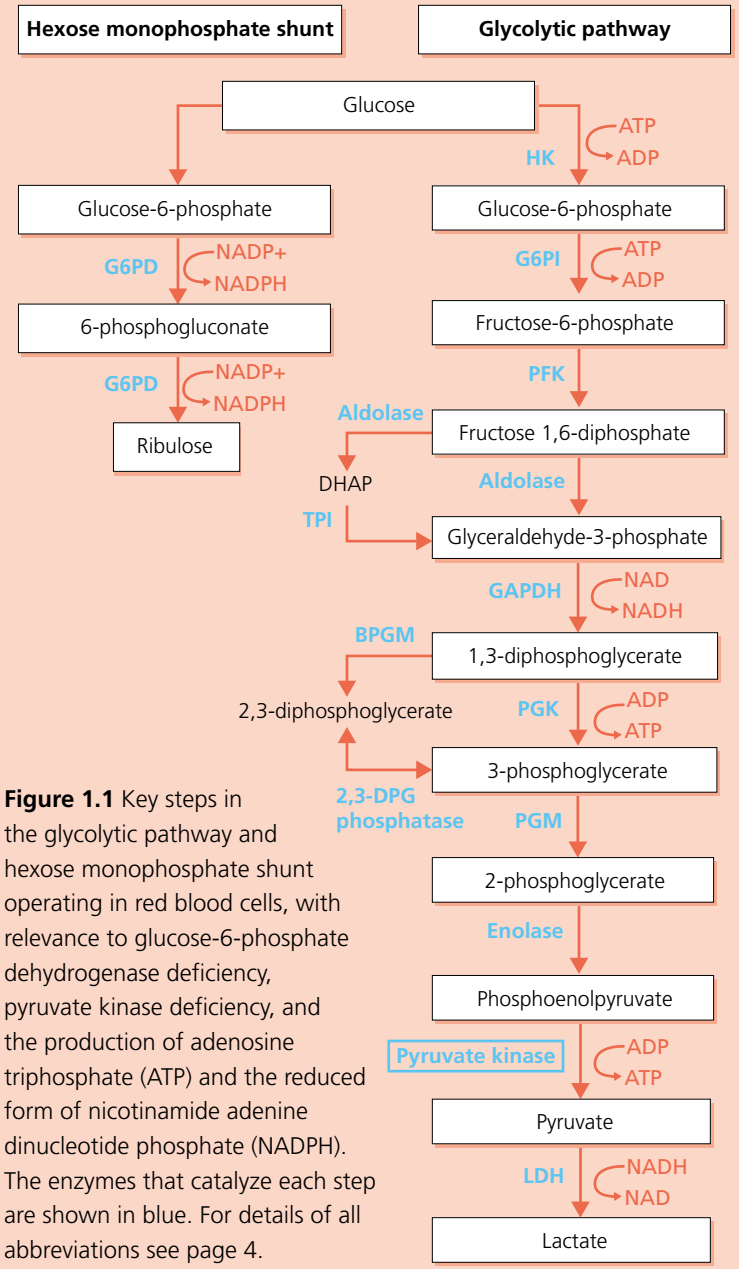


Figure 1.1 Key steps in the glycolytic pathway and hexose monophosphate shunt operating in red blood cells, with relevance to glucose-6-phosphate dehydrogenase deficiency, pyruvate kinase deficiency, and the production of adenosine triphosphate (ATP) and the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH). The enzymes that catalyze each step are shown in blue. For details of all abbreviations see page 4.

Hexose monophosphate shunt. The key product of the HMP shunt is NADPH, the reduced form of nicotinamide adenine dinucleotide phosphate. This cofactor plays a vital role in protecting RBCs from oxidant injury by assisting in the production of reduced glutathione. In RBCs, the reaction between hemoglobin and oxygen produces oxidants such as hydrogen peroxide and superoxide anion (O_2^-). If allowed to accumulate, these oxidants will oxidize hemoglobin and other proteins, leading to a loss of function and ultimately cell death. Reduced glutathione is an intracellular reducing agent and is essential for the inactivation of these oxidants and thus for the protection of RBCs. The HMP shunt is the only source of NADPH in RBCs, and under conditions of increased oxidative stress the amount of glucose entering the shunt can rise significantly.

Despite their metabolic limitations, normal RBCs have a lifespan of 100–120 days in the circulation.

Impact of enzyme disorders on red blood cells

The consequences of enzyme disorders for RBCs are quite variable. Some will result in hemolytic disease manifesting solely as anemia, while others will manifest as a multisystem disorder, with hemolysis being just one feature of the disease. The most common enzyme disorder of RBCs in humans is glucose-6-phosphate dehydrogenase (G6PD) deficiency.[4] This X-linked disorder affects more than 400 million people globally and disrupts the conversion of glucose-6-phosphate to 6-phosphogluconate in the HMP shunt (see Figure 1.1). As a consequence, the production of NADPH by RBCs is impaired and their ability to withstand oxidant injury is diminished.

Most affected patients remain asymptomatic, unless exposed to an oxidant challenge. Oxidant stresses due to exposure to certain drugs, infections, chemicals (naphthalene in mothballs) or foods such as fava beans can cause episodic hemolytic anemia. Chronic hemolysis affects only a very small minority of people with this disorder.

In contrast to G6PD deficiency, hemolytic anemias resulting from disorders of glycolytic enzymes are relatively rare, affecting only a few thousand individuals worldwide. While abnormalities in virtually every enzyme involved in the glycolytic pathway have been described, PK deficiency is the most common associated with hemolysis, accounting for more than 90% of cases.

Pyruvate kinase deficiency impairs normal glucose metabolism, resulting in inadequate ATP (energy) production by RBCs. In severely affected RBCs, the reduced levels of ATP lead to cell injury, a loss of membrane plasticity and the formation of rigid RBCs, which are destroyed in the spleen and liver.[5,6]

In some PK-deficient patients with high reticulocyte counts, ATP levels may be normal or even elevated. Since reticulocytes retain mitochondria, PK-deficient reticulocytes are able to generate ATP by mitochondrial oxidative phosphorylation rather than relying solely on the glycolytic pathway. However, as reticulocytes mature into erythrocytes, they lose their mitochondria and thus their ability to conduct oxidative phosphorylation.

The availability of oxygen and glucose within the spleen is limited, restricting the ability of PK-deficient reticulocytes to produce ATP via oxidative phosphorylation. The most severely affected reticulocytes will be destroyed, either in the spleen or subsequently in the liver. Less severely affected reticulocytes will have a longer survival.[7] Splenectomy can result in robust, sustained reticulocytosis because severely PK-deficient reticulocytes are no longer subjected to the adverse conditions within the spleen.

PK deficiency also results in a build-up of 2,3-DPG, an intermediate product of the glycolytic pathway (see Figure 1.1). Increases of up to three times normal have been recorded. Increased levels of 2,3-DPG cause a rightward shift in the oxygen dissociation curve of hemoglobin, thus enhancing the delivery of oxygen to tissues.[8] Consequently, patients with PK deficiency anemia may tolerate lower levels of hemoglobin than people with other forms of anemia in which 2,3-DPG is not elevated.

Manifestations of glycolytic enzyme disorders

Most glycolytic enzyme disorders are inherited in an autosomal recessive manner (the exception is phosphoglycerate kinase deficiency, which is an X-linked disorder). Individuals who are heterozygous for a mutation almost always show no hematologic abnormalities, although the level of enzyme activity within their RBCs is reduced. Hemolysis was initially believed to occur only in individuals who were homozygous for an enzyme deficiency. It is now known that true homozygosity for a given mutant enzyme is less common than originally thought.

Instead, most individuals with hemolytic anemia due to a deficiency in a glycolytic enzyme are heterozygous for two different enzyme variants. This is reflected in the heterogeneity of the biochemical and clinical manifestations of glycolytic enzyme defects.[9]

The clinical manifestations of PK deficiency are discussed in Chapter 5. More generally, the hemolysis resulting from an enzyme deficiency can manifest as chronic anemia of varying severity, reticulocytosis and some degree of indirect hyperbilirubinemia. Hemolysis may be accelerated as a result of non-specific infections. Also, worsening of anemia can be due to transient aplastic crisis associated with parvovirus B19 infection (see page 49).

Most patients with hemolytic anemia are diagnosed in childhood. Frequently, the history will include neonatal jaundice, often requiring an exchange transfusion, and, rarely, kernicterus. Once the more common causes of chronic hemolytic anemia have been ruled out (i.e. hereditary spherocytosis or hemoglobinopathies; see Chapter 3), the possibility of a glycolytic defect should be considered.

Pyruvate kinase isozymes

Two different genes, *PKM2* and *PKLR*, encode four distinct PK isozymes (Table 1.1).[2] Different tissues contain different PK isozymes:

- PK-M2 is present in all tissues during fetal development. Tissue-specific PK isozymes appear as fetal maturation proceeds, but PK-M2 persists as the predominant isozyme in mature leukocytes, platelets, and lung, kidney, spleen and adipose tissue. It is also the major isozyme in erythroid precursors.
- PK-M1 is present in mature muscle and brain tissue.

TABLE 1.1

Pyruvate kinase genes and isozymes

Gene	Chromosomal location	Isozyme encoded
PKM2	Chromosome 15 (15q22)	PK-M2
		PK-M1
PKLR	Chromosome 1 (1q21)	PK-L
		PK-R

- PK-L is the predominant isozyme in hepatocytes.
- PK-R is present in mature erythrocytes.

Mutations of the *PKLR* gene are associated with PK deficiency (see Chapter 2). PK deficiency was originally thought to result from decreased production of a structurally normal enzyme. However, it is now known that abnormal proteins are produced, which differ in both their physical properties and their biochemical kinetics.

In one severe form of PK deficiency (PK Beppu), the PK-M2 isozyme persists in mature erythrocytes. While it has been suggested that this continued expression of PK-M2 may allow affected RBCs to survive, the presence of the PK-M2 isozyme in mature erythrocytes can confound the interpretation of enzymatic activity assays (see pages 41–2) used in the diagnosis of PK deficiency.

Key points – overview

- Normal mature red blood cells (RBCs) rely on the breakdown of glucose via the glycolytic pathway to produce energy.
- The hexose monophosphate shunt protects RBCs from oxidant damage.
- Pyruvate kinase (PK) deficiency affects the penultimate step of the glycolytic pathway, impairing the conversion of phosphoenolpyruvate to pyruvate, and reducing the amount of energy produced by RBCs.
- PK deficiency is inherited in an autosomal recessive manner and is the most common glycolytic defect associated with hemolysis.

References

1. Valentine WN, Tanaka KR, Miwa S. A specific erythrocyte glycolytic enzyme defect (pyruvate kinase) in three subjects with congenital non-spherocytic hemolytic anemia. *Trans Assoc Am Physicians* 1961;74:100–10.

2. Zanella A, Bianchi P, Fermo E. Pyruvate kinase deficiency. *Haematologica* 2007;92:721–3.

3. Grimes AJ, Meisler A, Dacie JV. Hereditary non-spherocytic haemolytic anaemia. A study of red-cell carbohydrate metabolism in twelve cases of pyruvate-kinase deficiency. *Br J Haematol* 1964;10:403–11.

4. Beutler E. G6PD deficiency. *Blood* 1994;84:3613–36.

5. Nathan, DG, Oski FA, Sidel VW, Diamond LK. Extreme hemolysis and red-cell distortion in erythrocyte pyruvate kinase deficiency. II. Measurements of erythrocyte glucose consumption, potassium flux and adenosine triphosphate stability. *N Engl J Med* 1965;272:118–23.

6. Nathan DG, Oski FA, Miller DR, Gardner FH. Life-span and organ sequestration of the red cells in pyruvate kinase deficiency. *N Engl J Med* 1968;278:73–81.

7. Mentzer WC Jr, Baehner RL, Schmidt-Schonbein H et al. Selective reticulocyte destruction in erythrocyte pyruvate kinase deficiency. *J Clin Invest* 1971;50: 688–99.

8. Oski FA, Marshall BE, Cohen PJ et al. The role of the left-shifted or right-shifted oxygen-hemoglobin equilibrium curve. *Ann Intern Med* 1971;74:44–6.

9. Keitt AS. Pyruvate kinase deficiency and related disorders of red cell glycolysis. *Am J Med* 1966;41:762–85.

Further reading

Tanaka KR, Zerez CR. Red cell enzymopathies of the glycolytic pathway. *Semin Hematol* 1990;27:165–85.

2 Epidemiology and etiology

Despite being the best studied of the glycolytic enzyme disorders, gaps remain in our understanding of pyruvate kinase (PK) deficiency. More than 600 cases of PK deficiency have been reported, mainly from Europe, the USA and Japan, but the disease occurs worldwide, and it is likely that many cases go unreported. This may be because affected individuals die before birth, or because cases are mild and do not require medical attention. Alternatively, some cases may not be properly recognized, or they may be misdiagnosed. Given that some cases may not present with any of the findings usually associated with the condition, there are likely to be more cases of PK deficiency than are published in the literature. Patient registries have now been developed for PK deficiency, which are helping to characterize the range of symptoms and complications arising from this disorder.

Prevalence of pyruvate kinase deficiency

PK deficiency occurs equally in men and women. A study in northern England observed a prevalence of 3.3 cases of PK deficiency per million in a mainly white population.[1] Gene frequency studies have estimated the prevalence of PK deficiency in the general white population to be 51 cases per million.[2] In Germany and the USA, it has been estimated that about 1% of the population are heterozygous for PK deficiency,[3] while a study from Hong Kong found that 3% of newborn infants were heterozygous for a particular PK variant.[4]

Because PK deficiency is an autosomal recessive disorder, individuals who are heterozygous for a single PK mutation will not show clinical signs of hemolysis or anemia. For this reason, prevalence estimates based on the observation of clinically affected patients will be substantially lower than those based on the prevalence of heterozygotes in a population.

The Amish community in Pennsylvania, USA, has a particularly high frequency of PK deficiency, associated with one specific mutation,[5] which can be traced back to a single immigrant couple. An increased incidence has also been reported in children from a polygamist community in the western USA, again associated with a particular mutation.[6]

Mutations associated with pyruvate kinase deficiency

As discussed in Chapter 1, mutations in the *PKLR* gene on chromosome 1 are responsible for PK deficiency.[7] More than 300 different mutations have been reported; most are very rare, occurring only once. Currently, about 25% of patients diagnosed with PK deficiency appear to have a previously unrecorded mutation.

Although many of the mutations associated with PK deficiency are rare, a few are recorded more frequently (Table 2.1).

The variability in how PK deficiency manifests is thought to reflect the heterogeneity of these causative mutations, as well as the fact that most individuals with PK deficiency will be compound heterozygotes (i.e. they will have a different mutation in each copy of the *PKLR* gene).

Approximately 70% of the mutations associated with PK deficiency are missense (M) mutations (i.e. a single nucleotide change resulting in the production of a different amino acid), while the others are more disruptive non-missense (NM) mutations – either nonsense or

TABLE 2.1

More common mutations responsible for pyruvate kinase deficiency

Mutation	Effect	Regions/communities in which it is found most commonly
1529G→A	510Arg→Gln	USA, Europe*
1456C→T	484Arg→Trp	Spain, Portugal, Italy†
1468C→T	490Arg→Trp	Eastern hemisphere countries
1436G→A	479Arg→His	Amish community
	Deletion exon 11	Romany (Gypsy) population

*Found in 30–40% of affected white individuals in these regions.
†Accounts for approximately 30% of cases in these countries.
Arg, arginine; Gln, glutamine; His, histidine; Trp, tryptophan.

insertional mutations, deletions (such as the deletion of exon 11 found in the Romany population), or splicing abnormalities (see pages 43–4).

Genotype–phenotype relationship. Current research is focusing on the relationship between specific mutations in the *PKLR* gene and the severity of hemolysis.[8] Given the sheer number of different mutations and the predominance of compound heterozygotes, studies of the relationship between genotype and phenotype have grouped patients by type of mutation (i.e. two missense mutations [M/M], one missense and one non-missense mutation [M/NM], or two non-missense mutations [NM/NM]). Findings so far suggest that individuals with more disruptive (non-missense) mutations are more severely affected; they have lower levels of hemoglobin and require more frequent transfusions and a greater total transfusion volume. They also have a higher rate of splenectomy and a higher frequency of iron overload (see pages 43–4).

Acquired pyruvate kinase deficiency

While almost all cases of PK deficiency are inherited and result from mutations in the *PKLR* gene, an acquired form of the deficiency can very rarely occur secondarily to other blood diseases, such as acute leukemia, myelodysplastic syndrome and refractory sideroblastic anemia. Acquired PK deficiency can also result from complications associated with chemotherapy.

Key points – epidemiology and etiology

- Gaps remain in our understanding of the epidemiology of pyruvate kinase (PK) deficiency because many cases are believed to go unreported.
- About one quarter of patients diagnosed with PK deficiency will have a previously unrecorded mutation in the *PKLR* gene.
- Approximately 70% of mutations associated with PK deficiency are missense mutations.
- Some mutations are seen more frequently in specific regions and populations.
- Acquired PK deficiency very rarely can occur secondarily to other blood diseases.

References

1. Carey PJ, Chandler J, Hendrick A et al. Prevalence of pyruvate kinase deficiency in a northern European population in the north of England. *Blood* 2000;96:4005–6.

2. Beutler E, Gelbart T. Estimating the prevalence of pyruvate kinase deficiency from the gene frequency in the general white population. *Blood* 2000;95:3585–8.

3. Tanaka KR, Paglia DE. Pyruvate kinase deficiency. *Semin Hematol* 1971;8:367–96.

4. Fung RH, Keung YK, Chung GS. Screening of pyruvate kinase deficiency and G6PD deficiency in Chinese newborn in Hong Kong. *Arch Dis Child* 1969;44:373–6.

5. Kanno H, Ballas SK, Miwa S et al. Molecular abnormality of erythrocyte pyruvate kinase deficiency in the Amish. *Blood* 1994;83:2311–16.

6. Christensen RD, Eggert LD, Baer VL, Smith KN. Pyruvate kinase deficiency as a cause of extreme hyperbilirubinemia in neonates from a polygamist community. *J Perinatol* 2010;30:233–6.

7. Zanella A, Fermo E, Bianchi P, Valentini G. Red cell pyruvate kinase deficiency: molecular and clinical aspects. *Br J Haematol* 2005;130: 11–25.

8. Grace RF, Bianchi P, van Beers EJ et al. The clinical spectrum of pyruvate kinase deficiency: data from the Pyruvate Kinase Deficiency Natural History Study. *Blood* 2018;Mar 16. doi: 10.1182/blood-2017-10-810796. [Epub ahead of print]

Further reading

Baronciani L, Magalhaes IQ, Mahoney DH Jr et al. Study of the molecular defects in pyruvate kinase deficient patients affected by nonspherocytic hemolytic anemia. *Blood Cells Mol Dis* 1995;21:49–55.

Lenzner C, Nurnberg P, Thiele BJ et al. Mutations in the pyruvate kinase L gene in patients with hereditary hemolytic anemia. *Blood* 1994;83:2817–22.

National Institutes of Health, National Center for Advancing Translational Sciences, Genetic and Rare Diseases Information Center. Pyruvate kinase deficiency. rarediseases.info.nih.gov/diseases/7514/pyruvate-kinase-deficiency, last accessed 14 May 2018.

Zanella A, Bianchi P. Red cell pyruvate kinase deficiency: from genetics to clinical manifestations. *Baillieres Best Pract Res Clin Haematol* 2000;13:57–81.

3 Differential diagnosis

Pyruvate kinase (PK) deficiency is a chronic hemolytic anemia associated with acute exacerbations, varying degrees of severity and the potential to be diagnosed at any age. The differential diagnosis includes a heterogeneous group of hemolytic disorders, both acquired and congenital (Figure 3.1).

Although most of these conditions are rare, the coexistence of two or more causes of hemolysis cannot be excluded without additional assessment, further complicating diagnosis.

Many confounding factors can also complicate the clinical picture (i.e. concomitant iron or vitamin deficits, dysmyelopoiesis, liver or renal disease, chronic inflammation), underlining the need for a comprehensive clinical and laboratory evaluation of a patient presenting with a hemolytic disorder.

Assessment

The patient's history and clinical examination are fundamental to assess whether the disease is more likely to be congenital or acquired, whether the hemolysis is chronic or acute, and whether there is evidence of extrahematologic signs.

A complete blood count, reticulocyte count and metabolic chemistry panel are the first steps of the diagnostic process. This blood work-up helps to define the degree of anemia and the associated symptoms. It also helps to determine whether intra- or extravascular hemolysis is more prevalent and identifies the presence of reticulocytosis, which is an indicator of bone marrow compensation. Hemolytic parameters may vary in the different conditions, thus guiding the differential diagnosis.[1]

If at any point in the diagnostic process (see Figure 3.1) the findings no longer suggest a congenital hemolytic anemia, then acquired causes should be reconsidered and vice versa.

Acquired hemolytic anemias

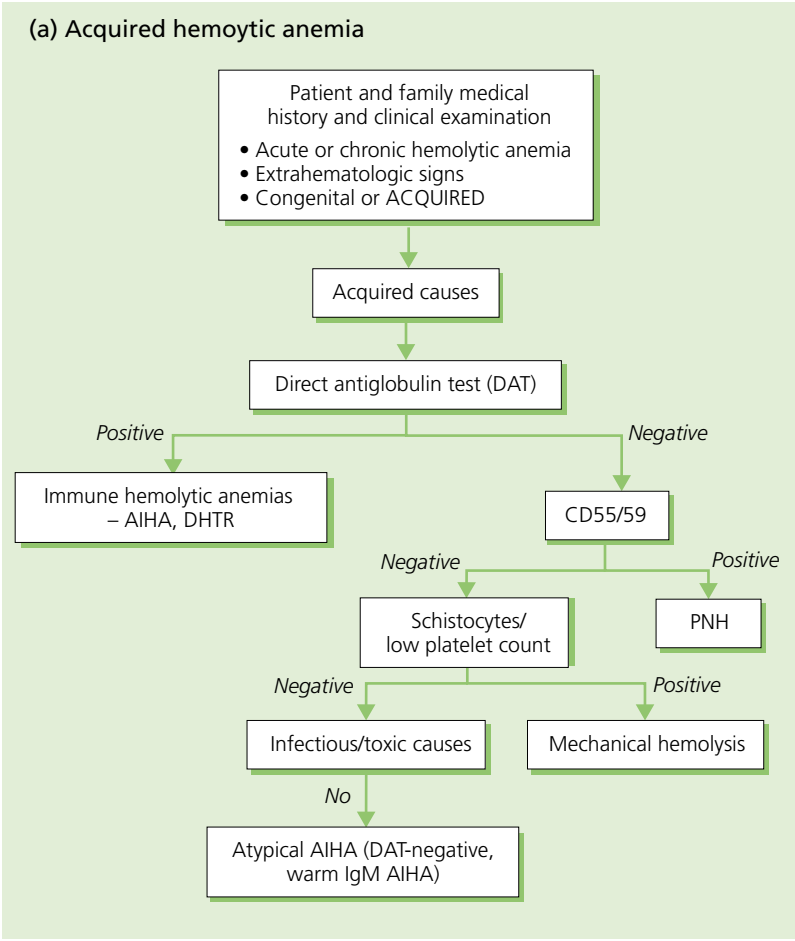
Autoimmune hemolytic anemias. The direct antiglobulin test (DAT, or Coombs test) is the cornerstone of the diagnosis of autoimmune hemolytic anemias (AIHAs).[2] The DAT reveals the presence of anti-erythrocyte antibodies on red blood cells (RBCs). Diffferent DAT methods have varying sensitivities/specificities, levels of automation and availability in laboratories.

The DAT tube utilizes the traditional agglutination technique, and is usually carried out using polyspecific reagents, although monospecific antisera (anti-immunoglobulin [Ig] G, anti-IgA, anti-IgM, anti-complement [C]) are advisable. Other methods include microcolumn DAT and solid-phase tests, which are suitable for automation and nowadays are available in most laboratories. The DAT tube is the most specific but least sensitive test, whereas microcolumn and solid-phase tests show reduced specificity but increased sensitivity. More sensitive techniques (i.e. flow cytometry, and enzyme-linked, radiolabeled and culture tests) are not routinely performed, and any positive result should be interpreted with caution given the low specificity of these techniques.

Types of autoimmune hemolytic anemia. Using the DAT, AIHAs are classified into warm forms (~70% of cases) in which the DAT is positive for IgG only or IgG plus C3d, cold agglutinin disease (~20% of cases) in which the DAT is positive for C3d only, and mixed forms in which the DAT is positive for IgG and C3d.

Hemolysis is primarily extravascular in the spleen in warm AIHAs, whereas it may also be intravascular in cold forms. Clinically, the level of RBC destruction by intravascular hemolysis has been calculated to be 200 mL of RBCs in 1 hour, more than ten times the level seen with extravascular hemolysis (Figure 3.2).

Confounding factors. A positive DAT result may occur because of the presence of alloantibodies in recently transfused patients, as a result of delayed hemolytic transfusion reactions, in hemolytic disease of the newborn and in a small number of healthy blood donors (< 0.1%) and hospitalized patients (0.3–8%) with no clinical evidence of AIHA. Moreover, a false-positive DAT may be observed after the administration of various therapeutics (i.e. intravenous immunoglobulins, Rhesus immune globulins and antithymocyte globulin), as well as in diseases with elevated serum globulins or paraproteins.



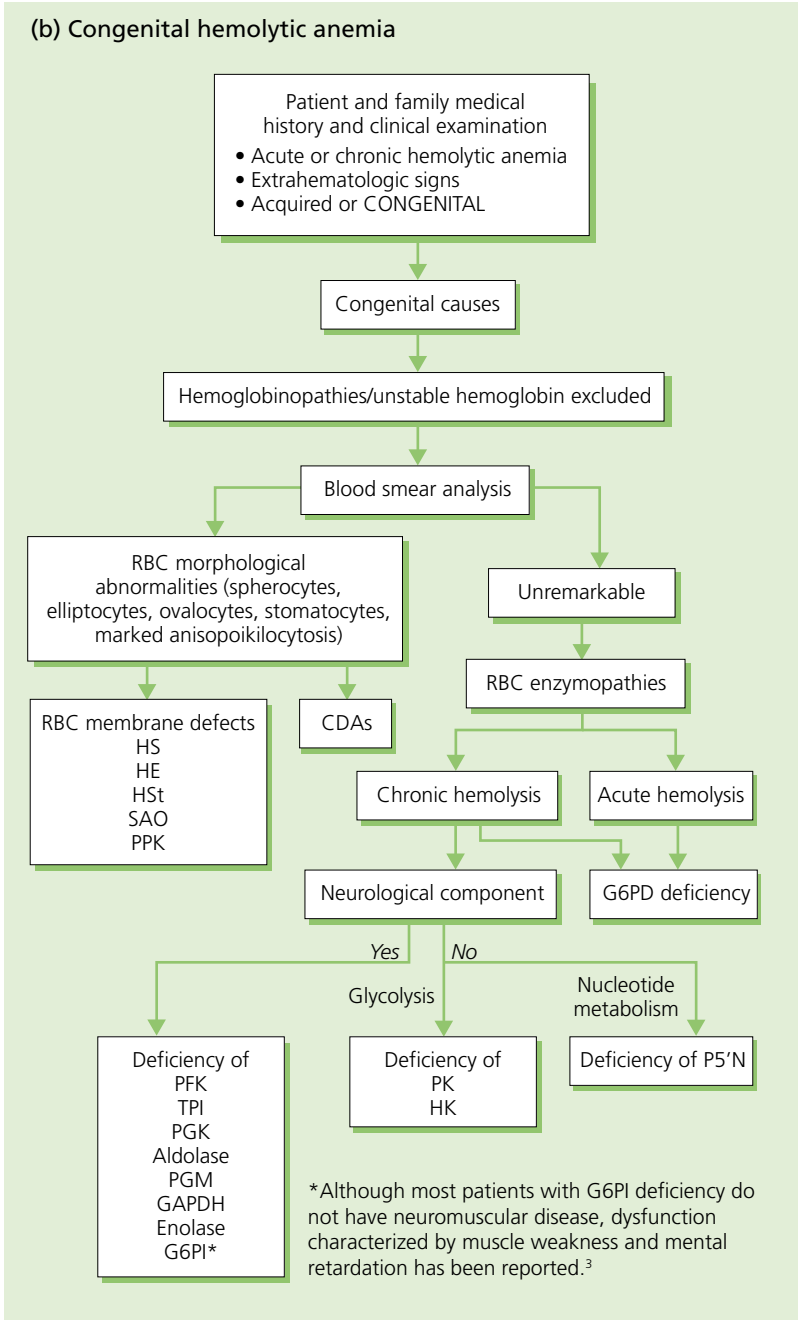


Figure 3.1 Diagnosis of (a) acquired and (b) congenital hemolytic anemias. AIHA, autoimmune hemolytic anemia; CDA, congenital dyserythropoietic anemia; DHTR, delayed hemolytic transfusion reactions; G6PD, glucose-6-phosphate dehydrogenase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; G6PI, glucose-6-phosphate isomerase; HE, hereditary elliptocytosis; HK, hexokinase; HS, hereditary spherocytosis; HSt, hereditary stomatocytosis; Ig, immunoglobulin; PGM, phosphoglycerate mutase; P5′N, pyrimidine 5-nucleotidase; PFK, phosphofructokinase; PGK, phosphoglycerate kinase; PK, pyruvate kinase; PNH, paroxysmal nocturnal hemoglobinuria; PPK, pyropoikilocytosis; RBC, red blood cell; SAO, Southeast Asian ovalocytosis; TPI, triosephosphate isomerase.

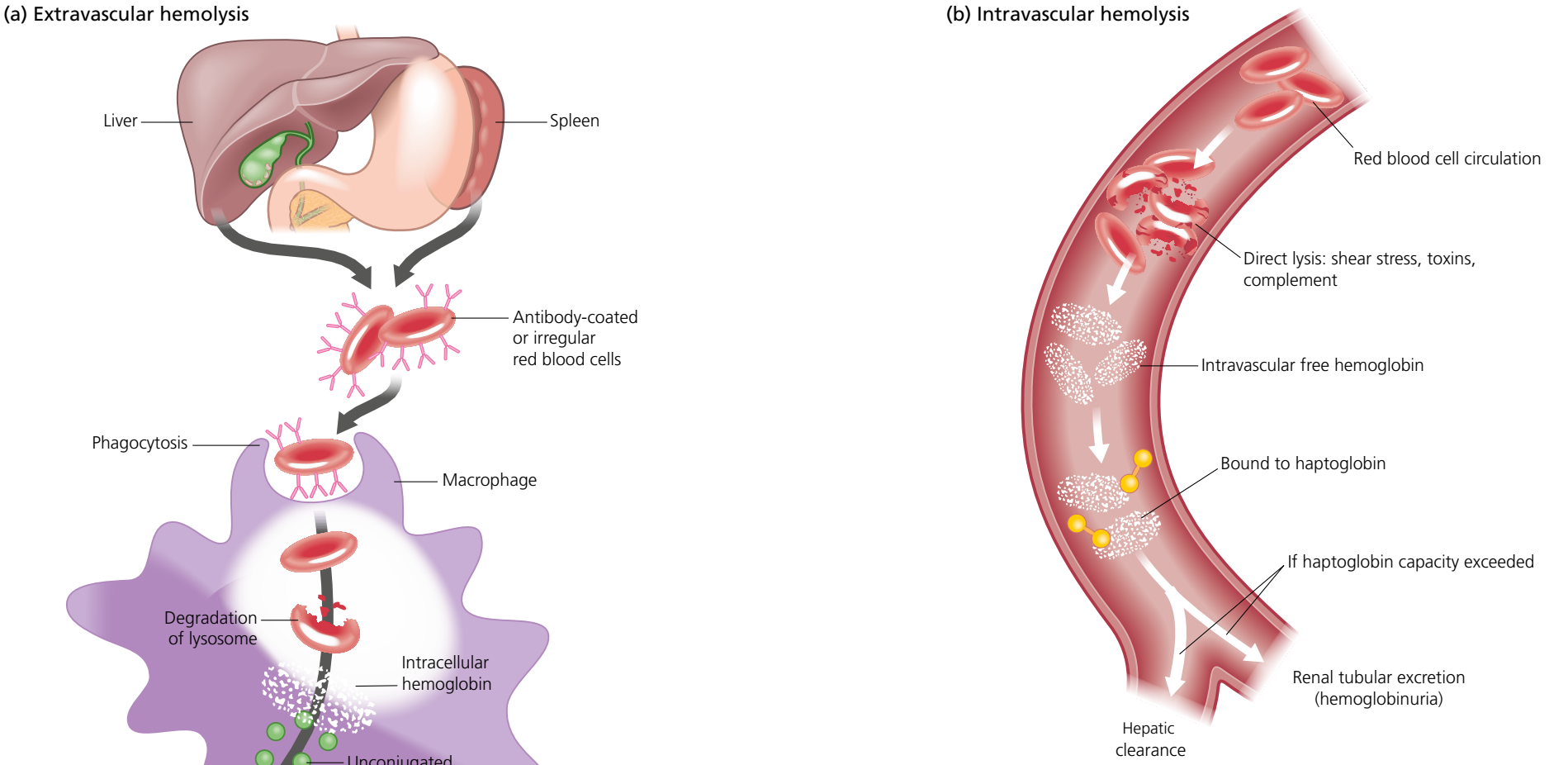


Figure 3.2 (a) Extravascular versus (b) intravascular hemolysis in autoimmune hemolytic anemia. In intravascular hemolysis the red blood cell destruction rate has been calculated as 200 mL of cells in 1 hour; in contrast, in extravascular hemolysis (which occurs mainly in the spleen, liver or lymphoid organs) it is about tenfold less (17 mL cells/hour). The clinical consequences are therefore quite different.

Conversely, in about 5% of AIHA (atypical forms) the DAT is negative by all the available methods, and the diagnosis is only made following extensive laboratory investigation to exclude other causes of hemolysis, and on the basis of the clinical response to steroid therapy.

Since AIHA may occur at any age, it should be considered a possibility in the differential diagnosis of congenital forms of hemolytic anemia.

Other acquired hemolytic anemias

There are several other causes of acquired hemolysis (see Figure 3.1a). The diagnostic work-up of all these conditions is beyond the scope of this book. However, alongside the patient's history and the serological and specific tests for the different diseases, it should be remembered that mechanical hemolysis is characterized by the presence of schistocytes (fragmented RBCs) on a blood smear examination, the early (and easy) detection of which may be crucial for prompt treatment and a consequent favorable prognosis.

Paroxysmal nocturnal hemoglobinuria (PNH) is a clonal disorder of hematopoietic stem cells characterized by chronic intravascular hemolysis, thrombosis, increased susceptibility to infections and bone marrow failure. The cellular abnormality originates from a mutation in the phosphatidylinositol glycan class A gene, resulting in a deficiency of glycosylphosphatidylinositol-(GPI)-anchored proteins, including CD55 and CD59. Deficiencies in these proteins, which inhibit the activation of complement, result in abnormally elevated susceptibility to complement-mediated lysis. The disease is easily diagnosed by flow cytometry analysis of GPI-deficient cells (PNH clones), and the clinical picture reflects the percentage of cells that are deficient. Three clinical subgroups have been identified:

- classic PNH (clone > 50%), dominated by intravascular hemolysis
- PNH in the setting of another bone marrow disorder (aplastic anemia, myelodysplastic syndrome or myelofibrosis)
- subclinical PNH (clone < 10% and no clinical or laboratory evidence of hemolysis).

Infectious agent-mediated hemolytic anemia. Several infectious agents can cause hemolytic anemia, including *Mycoplasma pneumoniae*, Epstein–Barr virus and cytomegalovirus. A hemolytic disease is often observed during upper respiratory tract infections, varicella infection, mumps, measles, Legionnaires' disease, visceral leishmaniasis and secondary syphilis. Direct destruction of RBCs may be mediated by a pathogen (*Plasmodium*, *Babesia* and *Bartonella* species) or by a toxin produced by a pathogen (*Clostridium perfringens*).

TABLE 3.1

Drugs that induce immune-mediated hemolytic anemia

Drug-independent induction	Drug-dependent induction
• Methyldopa	• Ceftriaxone
• Procainamide	• Cefotetan
• Ibuprofen	• Penicillin
• Diclofenac	• Piperacillin
• Fludarabine	• β-lactamase inhibitors
• Cladribine	• Other antibiotics

Microangiopathic forms of hemolytic anemia are characterized by mechanical destruction of RBCs, and include disorders such as thrombotic thrombocytopenic purpura, hemolytic uremic syndrome and disseminated intravascular coagulation. Some of these disorders are associated with infections, including Shiga toxin-producing *Escherichia coli*, *Shigella* and HIV. In addition, valvular rheumatic heart disease and infectious endocarditis may cause extremely turbulent blood flow resulting in mechanical intravascular hemolysis. This type of mechanical hemolysis is increasingly seen among recipients of prosthetic heart valves.

Drug-induced hemolytic anemia. Several drugs may be responsible for drug-induced immune-mediated hemolytic anemia by either a drug-independent or drug-dependent mechanism (Table 3.1).

Congenital hemolytic anemias

Membrane defects. Examination of a blood smear is fundamental to guiding the differential diagnosis of congenital hemolytic anemias. A typical morphology is observed in most membrane defects, such as hereditary spherocytosis, hereditary elliptocytosis, hereditary stomatocytosis and hereditary pyropoikilocytosis.

Hereditary spherocytosis is the most common form of membrane defect (Figure 3.3), with an estimated prevalence ranging from 1 in 2000 to 1 in 5000 in the general population. A dominant pattern of inheritance is seen in 75% of cases. Hereditary spherocytosis is due

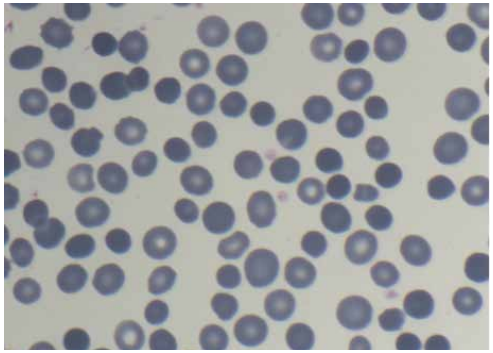


Figure 3.3 Typical morphology observed in hereditary spherocytosis; note the small spherical erythrocytes.

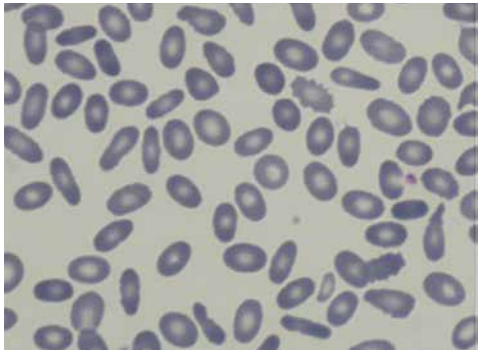
to a deficiency in the cytoskeletal proteins responsible for maintaining the biconcave morphology of RBCs. This defect results in reduced cell flexibility and premature destruction of spherocytes in the spleen. Typically, the osmotic fragility test, the acidified glycerol lysis test and flow cytometric analysis of eosin-5′-maleimide-labeled RBCs are positive.

The molecular defect is highly heterogeneous, involving the genes encoding for various membrane proteins (spectrin, ankyrin, band 3 and band 4.2). The defective protein can be detected by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, which is not mandatory for the diagnosis but may be useful for a differential diagnosis with other hematologic disorders that mimic hereditary spherocytosis, such as congenital dyserythropoietic anemia type II.

Hereditary elliptocytosis is characterized by a typical elliptic RBC shape (Figure 3.4), and is usually inherited in an autosomal dominant manner. It is due to defects in the interaction of alpha- and beta-spectrin (and rarely in band 4.1 and glycophorin). Usually it is clinically silent, with only mild hemolysis, with the exception of a variant form named hereditary pyropoikilocytosis (Figure 3.5). This form involves a severe functional defect in spectrin (the major cytoskeletal protein of the RBC cell membrane); it is an autosomal recessive disorder, and manifests as a severe hemolytic anemia with thermal instability of RBCs.

Hereditary stomatocytosis encompasses several autosomal dominant disorders, including dehydrated hereditary stomatocytosis or xerocytosis, overhydrated hereditary stomatocytosis, Southeast Asian ovalocytosis and familial pseudohyperkalemia. These are due

Figure 3.4 Typical morphology observed in hereditary elliptocytosis; note the elliptic red blood cells.



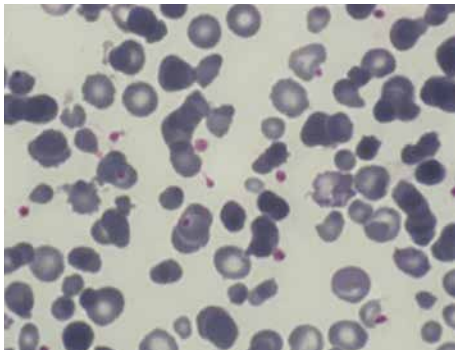
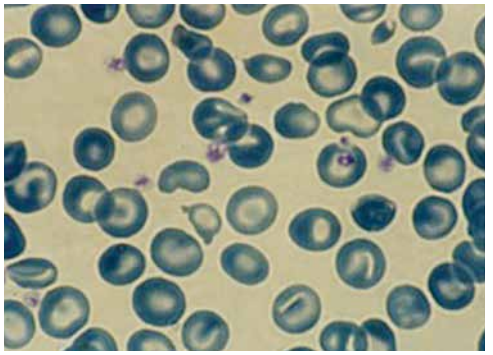


Figure 3.5 Typical morphology observed in hereditary pyropoikilocytosis, a severe variant of hereditary elliptocytosis. Large numbers of non-uniform fragmented erythrocytes, poikilocytes and microspherocytes are visible.

Figure 3.6 Typical morphology observed in hereditary stomatocytosis; 'mouth-like' erythrocytes can be seen.



to an abnormal permeability of the RBC membrane to monovalent cations (Na^+ and K^+), resulting in alterations in the cells' water content. Typical RBCs known as stomatocytes ('mouth-like' cells) are visible on blood smear examination (Figure 3.6). Several causative mutations are under investigation, including *RhAG* for overhydrated

hereditary stomatocytosis and *PIEZO1* and Gardos channel (*KCNN4*) for xerocytosis.

Congenital dyserythropoietic anemias. It is also worth considering the complex group of congenital dyserythropoietic anemias, which comprise several autosomal recessive disorders characterized by distinct morphological abnormalities of marrow erythroblasts. Typically, these anemias have some hemolytic features but an inadequate reticulocytosis. There are several causative mutations that classify the major subgroups, including *CDAN1*, *SEC23B*, *KIF23*, *KLF1* and *GATA-1*. Diagnosis of the congenital dyserythropoietic anemias requires bone marrow evaluation and molecular characterization in most patients.

Enzyme defects. When RBC morphology is unremarkable, a congenital hemolytic anemia due to an erythrocyte enzyme defect is highly likely. As explained in Chapter 1, RBCs need to continuously break down glucose via the glycolytic pathway and the hexose monophosphate shunt (HMP). Hereditary deficiencies of all HMP and glycolytic enzymes have been identified (see Figure 3.1b). With the exception of glucose-6-phosphate dehydrogenase deficiency (G6PD) and the rare 3-phosphoglycerate kinase (PGK) deficiency, both of which are X-linked, all other enzyme deficiencies are inherited in an autosomal recessive fashion.

Generally, all RBC enzymopathies are characterized by chronic hemolysis, with the exception of the most common subtypes of G6PD deficiency. Of note, most of the ultra-rare enzyme defects are also associated with a variable degree of neuromuscular pathology, particularly defects in phosphofructokinase, triosephosphate isomerase and PGK. In contrast, pyruvate kinase and hexokinase defects display the typical features of chronic hemolysis only.

A further enzyme defect worth mentioning is hereditary pyrimidine 5′-nucleotidase deficiency, which is the most frequent enzymopathy of RBC nucleotide metabolism causing non-spherocytic hemolytic anemia.

The diagnosis of all these conditions is based on the demonstration of reduced enzymatic activity and on the detection of mutations in the associated genes.

Hemolytic markers of disease

Disease-specific alterations in baseline hemolytic parameters can assist in differential diagnosis from the outset (Figure 3.7, Table 3.2).[1] For example, a rapid decrease in hemoglobin levels usually leads to symptoms (asthenia, tachycardia, dyspnea), suggesting acute hemolysis. This is more frequently observed in G6PD deficiency, PNH and warm AIHAs involving complement activation. Reticulocytosis and unconjugated hyperbilirubinemia are characteristic of extravascular hemolysis, whereas increased lactate dehydrogenase (LDH), free serum hemoglobin, hemoglobinuria and hemosiderinuria are typical of intravascular hemolysis, in which much higher levels of RBC destruction are seen (see Figure 3.7).

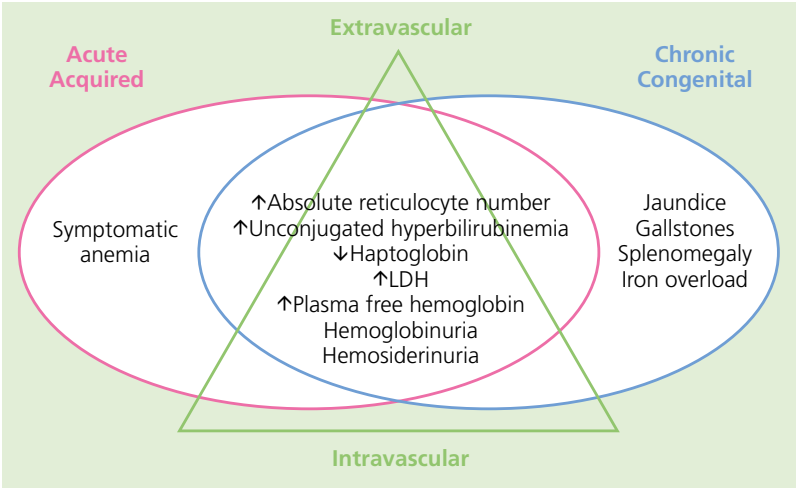


Figure 3.7 Differential alteration of hemolytic markers in the various hemolytic diseases. There is a progressive alteration of the hemolytic parameters from extravascular to intravascular hemolysis moving from the top to the bottom of the triangle. Reticulocytosis, increased unconjugated hyperbilirubinemia and haptoglobin consumption are more typical of extravascular hemolysis, whereas increased lactase dehydrogenase (LDH), plasma free hemoglobin, hemoglobinuria and hemosiderinuria are characteristic of intravascular hemolysis. Symptomatic anemia is generally (but not exclusively) observed in acute hemolysis and in acquired forms, whereas jaundice, gallstones, splenomegaly and iron overload are distinctive of chronic (mostly congenital) hemolytic anemias.

TABLE 3.2

Alterations in hemolytic parameters associated with various hemolytic diseases and confounding conditions

Parameter	Disease	Confounding conditions
Symptomatic anemia/ acute hemolysis	• Acute exacerbations in membrane/ enzyme defects, G6PD deficiency, PNH and AIHAs	• Other causes of anemia (blood loss, liver and renal insufficiency)
Reticulocytosis	• Mildly elevated in chronic/congenital hemolytic anemias • Dramatically raised in acute hemolytic crisis • Persistently increased in pyruvate kinase deficiency after splenectomy	• Hemorrhage • Pregnancy/delivery • Acclimation
Reticulocytopenia/ inadequate reticulocytosis	• Congenital dyserythropoietic anemias • AIHA with antibodies directed against bone marrow precursors	• Bone marrow disease (myelodysplasia, aplasia, leukemia, tumors) • Iron and vitamin deficiency • Renal disease • Infections
Hyperbilirubinemia	• All hemolytic diseases, particularly those with extravascular hemolysis	• Gilbert syndrome • Liver disease

(CONTINUED)

TABLE 3.2 (CONTINUED)

Alterations in hemolytic parameters associated with various hemolytic diseases and confounding conditions

Parameter	Disease	Confounding conditions
Increased LDH	• Four- to fivefold above the upper normal limit in conditions associated with the intravascular destruction of RBC (PNH, thrombotic microangiopathies, prosthetic valves) • Mildly elevated during acute hemolytic crisis due to infections in congenital forms of hemolytic anemia and in AIHA with complement activation • Slightly increased in extravascular hemolysis (warm AIHA and congenital hemolytic disorders)	• Diseases that involve cellular necrosis or increased tissue turnover (myocardial infarction, heart failure, hepatitis, extreme muscular effort and solid and hematologic tumors) • Vitamin B_{12} or folic acid deficiency
Reduced haptoglobin	• Reduced in all hemolytic conditions. It is the most sensitive marker, possibly being decreased even in the presence of normal hemoglobin levels	• Reduced in newborns, liver insufficiency, malnutrition and congenital hypo-haptoglobinemia • Increased in inflammatory diseases, cigarette smokers and nephrotic syndrome (possibly masking an underlying hemolysis)

(CONTINUED)

TABLE 3.2 (CONTINUED)

Alterations in hemolytic parameters associated with various hemolytic diseases and confounding conditions

Parameter	Disease	Confounding conditions
Hemoglobinuria and hemosiderinuria	• All conditions with marked intravascular hemolysis or acute hemolysis (PNH, G6PD deficiency, thrombotic microangiopathies)	• Prosthetic valves • Incompatible RBC transfusions, severe burns and infections
Increased ferritin	• Increased in all chronic hemolytic conditions (membrane defects and enzymopathies, chronic cold agglutinin disease and CDAs)	• Metabolic and inflammatory diseases • Hereditary hemochromatosis (even heterozygous) • Transfusions

AIHA, autoimmune hemolytic anemia; CDA, congenital dyserythropoietic anemia; G6PD, glucose-6-phosphate dehydrogenase; LDH, lactate dehydrogenase; PNH, paroxysmal nocturnal hemoglobinuria; RBC, red blood cell.

Reticulocytes (an index of bone marrow hematopoietic activity aimed at compensating for anemia) are mildly elevated in chronic/congenital hemolytic conditions, but dramatically raised in acute hemolytic crisis, and persistently increased in PK deficiency after splenectomy.

Conversely, compensatory reticulocytosis may be inadequate or absent in the presence of concomitant bone marrow involvement (myelodysplasia, hematologic malignancies, dyserythropoietic or bone marrow failure syndromes), iron and vitamin deficiency or renal disease (insufficient erythropoietin production) and in infections or autoimmune reactions against bone marrow precursors. Reticulocytes may also be increased in other conditions such as hemorrhage, pregnancy and delivery, and acclimation.

Hyperbilirubinemia during hemolysis is usually no more than 4 mg/dL. Higher values almost always imply concomitant impaired hepatic function and/or coexisting Gilbert syndrome (see Box 4.1).

Lactate dehydrogenase (mainly isozymes 1 and 2 of the five known isozymes) is:

- elevated four- to fivefold above the upper normal limit in disorders associated with the intravascular destruction of RBCs (PNH, thrombotic microangiopathies, prosthetic heart valves)
- mildly elevated in acute hemolytic crises resulting from infections in association with congenital anemias, and in AIHA with complement activation
- slightly increased in extravascular hemolysis (seen in warm AIHA and congenital hemolytic disorders).

Because of its ubiquitous distribution (heart, muscle, liver, brain), LDH can also increase in several conditions other than hemolysis that involve cellular necrosis or increased tissue turnover (i.e. myocardial infarction, heart failure, hepatitis, extreme muscular effort and solid and hematologic tumors). Moreover, LDH levels may be markedly increased in patients with vitamin B_{12} or folic acid deficiency because of ineffective erythropoiesis and premature RBC death.

Haptoglobin is synthesized by the liver and mainly acts as a scavenger of free serum hemoglobin. The haptoglobin–hemoglobin complexes are promptly cleared by the reticuloendothelial system, resulting in haptoglobin depletion. Haptoglobin is the most sensitive marker of hemolysis for both extravascular and intravascular RBC destruction, and can be decreased even in the presence of normal hemoglobin levels. Reduced levels are also observed in cases of liver impairment, malnutrition and congenital hypohaptoglobinemia. On the other hand, haptoglobin increases in inflammatory diseases, in people who smoke and in nephrotic syndrome, possibly masking an underlying hemolytic condition.

Ferritin is an intracellular protein that stores iron and releases it when required, acting as a buffer against iron deficiency and iron overload. Ferritin is increased in chronic hemolytic conditions (membrane defects

and enzymopathies, chronic cold agglutinin disease and congenital dyserythropoietic anemia). It is an acute phase protein and also increases in various metabolic and inflammatory diseases, in hereditary hemochromatosis (even in heterozygous patients), and as a result of transfusion support (which is often given to hemolytic patients).

Hemoglobinuria is the presence of abnormally high hemoglobin concentrations in the urine (giving it a dark color). It is usually due to RBC destruction in the circulation (intravascular hemolysis) and the release of free hemoglobin, which is filtered by the kidney.

Hemosiderinuria is due to hemosiderin bound to iron in the urine, and accounts for a 'brownish' color. Hemoglobin that is released into the bloodstream at levels exceeding the binding capacity of haptoglobin is then filtered by the kidney and reabsorbed in the proximal convoluted tubule. Here, its iron component is removed and stored as hemosiderin, and then excreted into the urine as the tubular cells are sloughed off. It is typically associated with marked intravascular hemolysis (PNH, G6PD deficiency) but is also found in reactions to incompatible RBC transfusions, severe burns and infections.

Leukopenia and thrombocytopenia. Among the various alterations in blood cell counts observed in hemolytic conditions, it is worth highlighting the possible reduction in leukocytes and platelets associated with vitamin B_{12} and folic acid deficiency, Evans syndrome, PNH-associated bone marrow failure syndromes, thrombotic microangiopathies and intravascular devices. In patients with congenital membrane or enzyme defects mild thrombocytopenia may be related to hypersplenism, whereas thrombocytosis may be observed after splenectomy.

Key points – differential diagnosis

- The differential diagnosis of pyruvate kinase deficiency includes a heterogeneous group of acquired and congenital hemolytic disorders.
- The direct antiglobulin test (Coombs test) is the cornerstone of the diagnosis of autoimmune hemolytic anemias. False-positive and false-negative results should be taken into account.
- Several infectious, microangiopathic, mechanical, toxic and pharmacological causes of acquired hemolytic anemias should also be considered.
- Patient history and blood smear examination are fundamental in the diagnosis of congenital membrane defects, including hereditary spherocytosis, elliptocytosis and stomatocytosis.
- Congenital dyserythropoietic anemias and hereditary pyropoikilocytosis are recessive anemias to be considered and diagnosed mainly by molecular analysis.
- A complex group of enzyme defects (mostly recessive) are diagnosed by testing the enzymatic activity in the peripheral blood and by confirming the causative mutation at a molecular level (homozygous or compound heterozygous).

References

1. Barcellini W, Fattizzo B. Clinical applications of hemolytic markers in the differential diagnosis and management of hemolytic anemia. *Dis Markers* 2015;2015:635–70.

2. Barcellini W. Immune hemolysis: diagnosis and treatment recommendations. *Semin Hematol* 2015;52:304–12.

3. Manco L, Bento C, Pereira J et al. Hereditary nonspherocytic hemolytic anemia caused by red cell glucose-6-phosphate isomerase (GPI) deficiency in two Portuguese patients: clinical features and molecular study. *Blood Cells Mol Dis* 2016;60:18–23.

4 Diagnosis of pyruvate kinase deficiency

The diagnosis of pyruvate kinase (PK) deficiency begins with an assessment of a patient's history, clinical signs/symptoms and laboratory markers of chronic hemolytic anemia. However, clinical symptoms can vary greatly between affected individuals.[1] Most importantly, diagnosis is assisted by evidence of reduced PK enzyme activity together with the detection of compound heterozygous and homozygous mutations in the *PKLR* gene.[2–5]

As would be expected, patients with more severe anemia are diagnosed at a younger age, but PK deficiency can also be diagnosed later into adulthood.

PK deficiency may be difficult to diagnose for several reasons:

- The clinical heterogeneity of the disease, which ranges from asymptomatic cases to life-threatening neonatal anemia, and even hydrops fetalis.
- As an autosomal recessive disorder there may be no family history to assist clinicians (i.e. the parents of an affected individual are most often hematologically normal and there may be no affected siblings).
- There are difficulties with the performance and interpretation of assays for PK enzymatic activity.
- Many different mutations in the *PKLR* gene have been associated with PK deficiency and about one-quarter of patients will have a novel mutation.
- The differential diagnoses include several other forms of hemolytic anemia, which may be unfamiliar to clinicians (see Chapter 3).
- Hemolytic markers can be difficult to interpret and can be confounded by other conditions (see Table 3.2, page 32).

It is likely that many cases of PK deficiency remain unrecognized. In fact, there is a five- to 15-fold difference between the prevalence of PK deficiency as estimated by genetic models and the number of diagnosed cases. Consequently, the exact prevalence of PK deficiency is largely unknown.

Clinical signs and symptoms

The clinical complications of PK deficiency are outlined below and discussed in more detail in Chapter 5. They comprise the usual hallmarks of lifelong chronic hemolysis, with anemia, splenomegaly, jaundice and gallstones being the most frequent (Table 4.1). Early onset of symptoms is usually associated with a more severe clinical course, and infants and young children can be transfusion dependent shortly after birth.

Anemia is relatively constant in young children through adulthood. Exacerbations of anemia are frequently observed in association with acute infections, stress and pregnancy, and commonly require transfusions. As discussed in Chapter 1, anemia occasionally may be well tolerated in patients with PK deficiency because of the increased levels of 2,3-diphosphoglycerate in red blood cells (RBCs), resulting in a rightward shift in the hemoglobin–oxygen dissociation curve.

TABLE 4.1

Main clinical signs and symptoms associated with pyruvate kinase deficiency[1,4]

Sign/symptom	Patients reporting (%)
Anemia	90–95
Splenomegaly	80–85
Neonatal jaundice	59–90
Jaundice	40–70
Gallstones	30–45
Aplastic crisis	2–14
Bone deformities	9
Extramedullary erythropoiesis	9
Slowed/delayed puberty	8
Hyperpigmentation	6
Leg ulcers, pulmonary hypertension	2–3

Splenomegaly is present in almost all patients with PK deficiency, with a variable degree of enlargement. In total, 30–60% of patients (generally those most severely affected and/or dependent on transfusions) opt for splenectomy, often in an attempt to decrease the transfusion burden or improve the baseline hemoglobin. However, about 14% of these patients will remain transfusion dependent following splenectomy.[1]

Jaundice is observed in a large number of cases, and neonatal jaundice is a common finding, requiring exchange transfusion in about 50% of patients.

Gallstones are detected in patients of all ages, with a median age of diagnosis of 15 years. Overall, gallstones are diagnosed in about 50% of patients and occur even after splenectomy as a result of ongoing hemolysis.

Iron overload is frequently observed, even in patients who do not receive regular transfusions. The cause is unclear but may involve a degree of ineffective erythropoiesis, and co-inheritance of hereditary hemochromatosis mutations.

Laboratory testing

Red blood cell morphology in PK deficiency is unremarkable on a peripheral blood smear, generally displaying some degree of anisocytosis, poikilocytosis and polychromatophilia. A variable proportion of echinocytes (5–20%) is occasionally observed, more commonly after splenectomy.

Hemoglobin levels in patients with PK deficiency typically range from 6–12 g/dL[6] and increase by about 1.6 g/dL after splenectomy.[1]

Reticulocyte count is usually elevated (by 4–11%), as in other hemolytic conditions. In PK-deficient patients, splenectomy usually results in a conspicuous rise in the reticulocyte count. It is important to be aware that reticulocytopenia/inadequate reticulocytosis may be a sign of an aplastic crisis, usually a consequence of parvovirus infection.

Hyperbilirubinemia. The median indirect hyperbilirubinemia in PK deficiency is about 3.5 mg/dL. Hyperbilirubinemia during hemolysis is usually no more than 4 mg/dL, and higher values almost always imply coexisting Gilbert syndrome (Box 4.1).

BOX 4.1

Gilbert syndrome

- An autosomal recessive inherited abnormality
- Affects 5–15% of the population
- Reduces production of the bilirubin-specific form of uridine 5′-diphosphoglucuronosyl transferase, which converts unconjugated bilirubin to conjugated bilirubin in the liver
- Results in a build up of unconjugated bilirubin in the blood, leading to jaundice
- Affected individuals often experience a worsening of their everyday jaundice around the time of puberty

Ferritin is frequently increased, even in patients who do not receive regular transfusions. About 50% of patients with PK deficiency have values over 1000 ng/mL or are on chelation therapy.

Lactate dehydrogenase, which is mainly a marker of intravascular hemolysis, is not usually elevated in PK deficiency.

Assessment of enzyme activity

PK enzymatic activity is usually determined in RBC lysates by spectrophotometric assay. Activity is calculated from the rate of pyruvate production, linked to the oxidation of NADH (the reduced form of nicotinamide adenine dinucleotide). The decrease in optical density that occurs as NADH is oxidized is measured by a spectrophotometer at 340 nm. The assay takes 2–3 hours to perform and is not expensive.

However, there is no clear correlation between the severity of the clinical picture and PK activity[6] as determined by the assay. In fact, patients with only mild clinical signs may display severely reduced PK activity and vice versa.

False-positive results suggesting PK deficiency may be due to poor sample storage. For this reason a normal control sample stored under the same conditions should also be tested. PK enzyme activity may be considered stable at 4°C for up to 21 days after blood collection, although a maximum of 14 days of storage is recommended.

It is also important to remember that false-normal levels of PK activity may be due to the presence of increased numbers of reticulocytes, since most of the enzymatic activity of RBCs is strongly influenced by cell age; it is greater in young cells. Other factors that may give rise to false-normal levels of PK activity in PK-deficient patients include incomplete platelet and leukocyte removal during preparation of a sample for testing, compensatory expression of the PK-M2 isoenzyme (see page 14) and recent transfusions. It is recommended that testing of PK activity is delayed until at least 40–60 days after a transfusion.

A further potential complicating factor is the existence of kinetically abnormal mutant PK enzymes that display normal or even increased activity in vitro but are ineffective in vivo.

Decreased PK activity may be found in heterozygous carriers (although the levels of reduction are generally less). Therefore, genotyping of the *PKLR* gene is strongly recommended to confirm a diagnosis of PK deficiency, particularly before splenectomy, which is contraindicated in some forms of chronic hemolytic anemia, such as hereditary stomatocytosis.

DNA sequence analysis of the *PKLR* gene

The *PKLR* gene encoding erythrocyte PK is located on chromosome 1q21.[7] It consists of 12 exons and is approximately 9.5 kb in size. To date, more than 300 mutations in *PKLR* have been associated with PK deficiency (see page 17).

With the advent of next-generation sequencing (NGS) techniques for diagnostic purposes, *PKLR* is typically included in gene panels designed for the diagnosis of hereditary hemolytic anemias.[8] DNA analysis offers obvious advantages compared to biochemical testing.

- It requires smaller sample volumes.
- Samples are easier to handle and ship.
- There is no interference from transfused RBCs.
- It is suitable for prenatal diagnosis.

However, not all the mutations detected by DNA analysis can be immediately classified as causative until their pathogenic nature is confirmed by other functional tests. In fact, there are patients who are homozygous or compound heterozygous for *PKLR* mutations who show normal PK activity.

Genetic testing will develop further with the use of whole-genome sequencing, although it will still be limited by the ability to interpret bioinformatic data.

At present, most reference centers screen for PK deficiency by measuring PK enzymatic activity and then confirm suspected PK deficiency by DNA sequence analysis of the *PKLR* gene. In a few centers, screening for PK deficiency is initially performed by NGS panels and then (in the case of an identified novel mutation) confirmed by PK enzymatic activity.

Genotype–phenotype relationship

The first attempts to correlate the mutations found in the *PKLR* gene with the clinical phenotype of affected patients were carried out by analyzing the few known homozygous patients and by studying larger series of compound heterozygous cases grouped according to their clinical phenotype.[3] In these studies, patients were divided into severe (hemoglobin [Hb] < 8 g/dL and/or > 50 transfusions), moderate (Hb 8–10 g/dL and/or > 10 transfusions) and mild (Hb ≥ 10 g/dL) phenotypes.

The severe phenotype was commonly associated with disruptive mutations (i.e. stop codon, frameshift, splicing and large deletions) and with missense mutations directly involving the active site or affecting protein stability. Of these mutations, homozygosity for the 994G→A missense mutation (which results in the production of serine instead of glycine) was associated with very severe hemolytic anemia. The very few patients with homozygous 'null' mutations (resulting in the absence of a functional protein) displayed intrauterine growth retardation, severe anemia at birth, transfusion dependence and, in rare cases, intrauterine death or death in the first days of life.

As shown in Table 2.1 (page 17), the most frequent mutation associated with the moderate phenotype is 1529G→A (commonly found in white American and northern European populations and

resulting in the production of glutamine instead of arginine) in either homozygous or compound heterozygous presentations.

Molecular analysis of the mild phenotype showed a predominance of mutation 1456C→T (most frequent in southern Europe and resulting in the production of tryptophan rather than arginine). This mutation resulted in a mild phenotype even in association with disruptive mutations, such as frameshift or stop codon mutations.

More recently, the genotype–phenotype correlation has been investigated by the production and characterization of recombinant mutant proteins and their comparison with the wild-type PK enzyme.[5] This approach allowed the definition of the effects of amino acid replacements on the stability and kinetic properties of PK. However, the clinical manifestations of PK deficiency also include genetic post-translational or epigenetic modifications, along with ineffective erythropoiesis and differences in splenic function, making it complex to interpret these in vitro findings.

Finally, the genotype–phenotype relationship has been investigated in the international multicenter PK deficiency registry, which includes retrospective and prospective data from 254 patients, the largest series reported to date.[1] As discussed in Chapter 2 (page 17), patients were grouped as having two missense (M/M), one missense/one non-missense (M/NM) or two non-missense (NM/NM) mutations. Non-missense mutations, which were disruptive changes, included nonsense, frameshift, inframe small indel, large deletions and splicing mutations. The NM/NM group had a more severe phenotype, with earlier diagnosis, more severe anemia, a higher rate of splenectomy, a greater need for transfusion and higher ferritin levels. Notably, there was no evidence of an association between PK enzyme activity and the genotype.

Key points – diagnosis

- Pyruvate kinase (PK) deficiency should be considered in patients with signs/symptoms and laboratory findings of chronic hemolytic anemia (including those with mild anemia), reticulocytosis, jaundice, unconjugated hyperbilirubinemia, gallstones and splenomegaly.
- The diagnosis of PK deficiency is made by measuring PK enzymatic activity in red blood cell lysates by spectrophotometric assay.
- The diagnosis should be confirmed by the demonstration of known causative *PKLR* mutations in homozygous or compound heterozygous presentations.

References

1. Grace RF, Bianchi P, van Beers EJ et al. The clinical spectrum of pyruvate kinase deficiency: data from the Pyruvate Kinase Deficiency Natural History Study. *Blood* 2018;Mar 16. doi: 10.1182/blood-2017-10-810796. [Epub ahead of print].

2. Bianchi P, Zanella A. Hematologically important mutations: red cell pyruvate kinase (Third update). *Blood Cells Mol Dis* 2000;26:47–53.

3. Zanella A, Bianchi P. Red cell pyruvate kinase deficiency: from genetics to clinical manifestations. *Baillieres Best Pract Res Clin Haematol* 2000;13:57–81.

4. Zanella A, Fermo E, Bianchi P, Valentini G. Red cell pyruvate kinase deficiency: molecular and clinical aspects. *Br J Haematol* 2005;130:11–25.

5. Zanella A, Fermo E, Bianchi P et al. Pyruvate kinase deficiency: the genotype-phenotype association. *Blood Rev* 2007;21:217–31.

6. Tanaka KR, Paglia DE. Pyruvate kinase deficiency. *Semin Haematol* 1971;8:367–96.

7. Satoh H, Tani K, Yoshida MC et al. The human liver-type pyruvate kinase (PKL) gene is on chromosome 1 at band q21. *Cytogenet Cell Genet* 1988;47:132–3.

8. Fermo E, Vercellati C, Marcello AP et al. Use of next generation sequencing panel to clarify undiagnosed cases of hereditary hemolytic anemias. *Blood* 2017;130:3480.

Further reading

Grace RF, Zanella A, Neufeld EJ et al. Erythrocyte pyruvate kinase deficiency: 2015 status report. *Am J Hematol* 2015;90:825–30.

5 Complications and monitoring

Pyruvate kinase (PK) deficiency can be associated with a range of complications (Table 5.1).[1] Although complications are more frequent in patients with severe anemia and frequent transfusions, the likelihood of a given complication in an individual patient is not predictable based on hemoglobin and transfusion burden and thus all patients with PK deficiency require regular monitoring (Table 5.2).

Main complications

Anemia. The hemolytic anemia due to PK deficiency varies from mild to severe, with hemoglobin levels typically in the range of 6–12 g/dL. Reticulocyte counts may be inappropriately low in children or increased as a reflection of the hemolysis. After splenectomy, reticulocyte counts will paradoxically rise to 30–70% of red blood cells (RBCs).

In patients who receive regular blood transfusions, complete blood counts should be evaluated before transfusion. In those patients who

TABLE 5.1

Main complications associated with pyruvate kinase deficiency

- Anemia
  - Hyperhemolytic episodes
  - Aplastic crisis
- Jaundice and gallbladder disease
  - Gallstones
- Splenomegaly
- Iron overload
- Extramedullary hematopoiesis
- Osteopenia
- Pulmonary hypertension
- Leg ulceration

TABLE 5.2

Monitoring tests and suggested frequency*

Test	Timing
Complete blood counts, reticulocyte count	Annually and as needed for symptoms of worsening anemia
Total/indirect bilirubin	Annually and as needed for symptoms of worsening anemia and/or jaundice
Ferritin	Annually for iron monitoring or more frequently if on chelation or therapeutic phlebotomy for iron removal
MRI (FerriScan)	MRI, if available, should be used for assessing iron overload in all patients with PK deficiency, including those who have never been transfused. Although cardiac hemosiderosis is uncommon in PK deficiency, both cardiac and hepatic iron should be evaluated. In non-transfused patients, the timing of the first MRI may be delayed until sedation is unnecessary and may also be delayed until the ferritin reaches a threshold determined by the physician (e.g. ferritin > 500 ng/mL). The frequency of MRI in non-transfused patients will be based on the initial findings and correlation with ferritin
25-hydroxyvitamin D level	Annually
Bone density scan	Baseline assessment, with the timing of follow-up assessments depending on initial findings and interventions to optimize bone health. First assessment suggested in late adolescence or early adulthood
Abdominal ultrasound	Screen for gallbladder disease before splenectomy and for new abdominal symptoms or worsening jaundice. Screening ultrasounds in the absence of symptoms are at the discretion of the treating clinician but could be considered, particularly in the setting of co-inherited Gilbert syndrome

(CONTINUED)

TABLE 5.2 (CONTINUED)

Monitoring tests and suggested frequency*

Test	Timing
Viral screen (HIV, hepatitis A, B, C)	Annual infection screening is indicated in patients who have received transfusions
Echocardiogram	Consider in individuals age > 30 years, before pregnancy and at any age in individuals with symptoms suggestive of poor cardiac function and/or pulmonary hypertension
Hormone evaluation	Individuals with iron loading should be screened for endocrinopathies related to the thyroid, sex hormones and pancreas/glycemia. The HbA_{1C} test for diabetes will not be accurate in the setting of hemolytic anemia; assessing fructosamine levels is recommended for screening for diabetes

*The types and frequency of tests for monitoring may differ between patients. Additional medications, such as iron chelators, will require additional monitoring.

do not receive transfusions on a regular basis, complete blood counts and a reticulocyte count should be monitored at least annually and with any significant worsening of baseline symptoms.

Patients who receive transfusions, regularly or irregularly, should have annual viral screening.

There is no relationship between the measured PK enzyme activity level and the degree of hemolysis;[2] once performed to confirm the diagnosis of PK deficiency, this test does not need repeating over time.

Hemolytic episodes or crises develop in the setting of stressors or triggers of hemolysis, which are most often due to infections and, therefore, occur more frequently in childhood. Pregnancy can also be a common hemolytic trigger. Symptoms include:

- fatigue
- pallor
- scleral icterus and jaundice associated with significant hemolysis
- indirect hyperbilirubinemia
- hemoglobinuria.

Spleen size may also increase during hemolytic episodes.

Aplastic crises may complicate the clinical course of PK deficiency and are usually associated with infection with parvovirus B19. This infection results in a sudden temporary cessation of erythropoiesis and dramatic decreases in both hemoglobin concentration and reticulocyte count. Patients with PK deficiency who have an acute parvovirus infection will usually require a transfusion, even if they have never been previously transfused. Parvovirus infection will occur only once in a patient's lifetime.

Jaundice and gallbladder disease. In patients with hemolytic anemia, the degree of indirect hyperbilirubinemia is determined both by the severity of hemolysis and also by modifiers of bilirubin metabolism. Nearly every patient with PK deficiency will have an elevated indirect bilirubin level (Figure 5.1). Co-inheritance of Gilbert syndrome (see Box 4.1) will cause a significantly more elevated bilirubin level (i.e. indirect bilirubin > 5 mg/dL) than would otherwise be suggested by the hemolytic rate. However, when suspected, the clinical utility of testing for Gilbert syndrome is not clear.

Although splenectomy improves the anemia for most people with PK deficiency, it does not resolve the issue of jaundice or scleral icterus as the hemolytic process continues despite splenectomy. Thus, splenectomy should not be pursued to improve jaundice in patients with PK deficiency.

Gallstones are a frequent complication in patients of all ages with PK deficiency because of the increased unconjugated bilirubin related to hemolysis. The risk of bilirubin gallstones is lifelong because hemolysis will continue after splenectomy. Therefore cholecystectomy should be considered concurrently with splenectomy, even in individuals who do not have evidence of gallstones, as almost half of patients who have a splenectomy only will ultimately go on to have a cholecystectomy.[1]

Splenomegaly. Many individuals with PK deficiency develop splenomegaly. Splenomegaly can worsen during hemolytic episodes and/or concurrent viral infections. Hypersplenism can occur in the setting of splenomegaly and should be suspected in patients with increasing transfusion burden and/or mild thrombocytopenia and/or neutropenia.

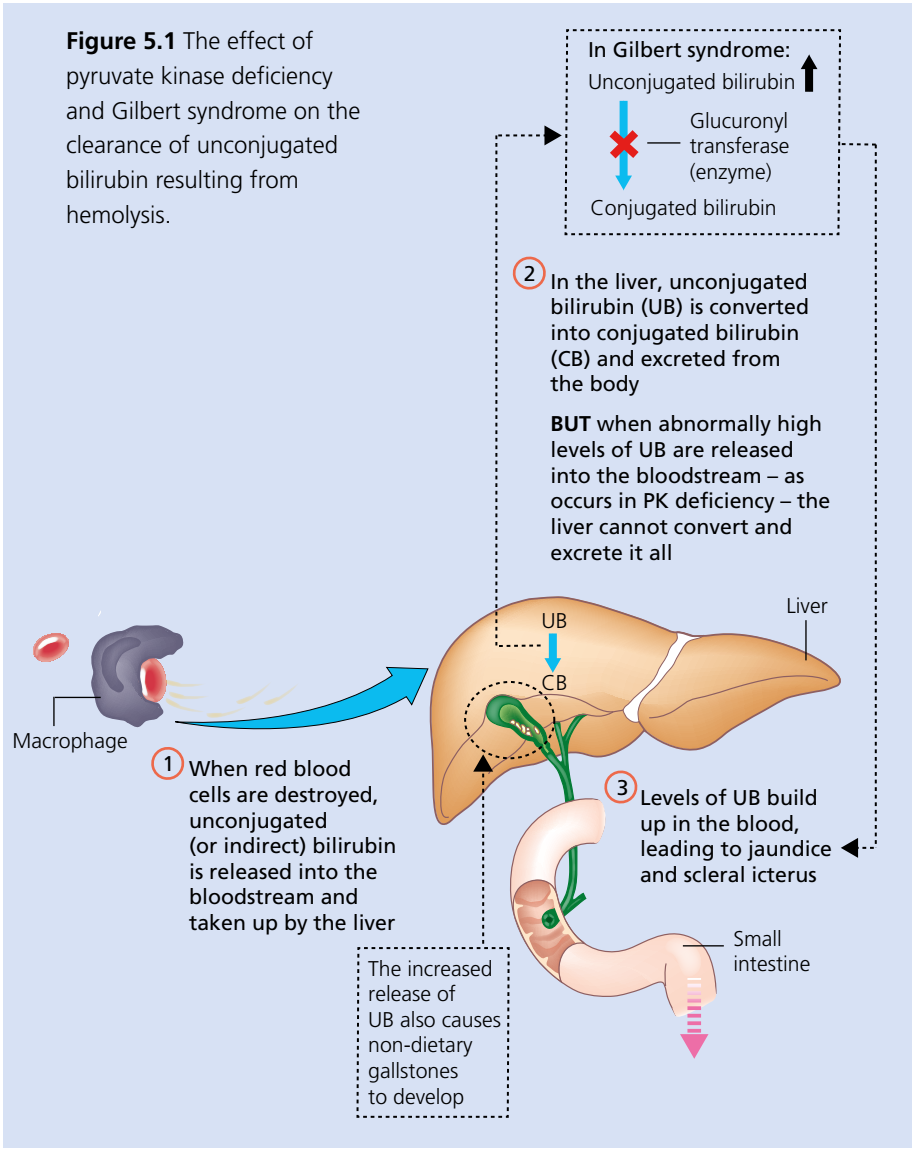


Figure 5.1 The effect of pyruvate kinase deficiency and Gilbert syndrome on the clearance of unconjugated bilirubin resulting from hemolysis.

Splenic injury following high-impact trauma to the abdomen may be a particular risk in patients with palpable splenomegaly, and contact sports should be avoided by patients with marked splenomegaly.

Although splenomegaly is typical in PK deficiency, a normal spleen size does not preclude the condition or the role of the hypoxic splenic environment in hemolysis. In severe anemia, splenectomy may be beneficial even with a normal spleen size.

Iron overload. Transfusion-associated hemosiderosis is a predictable complication of chronic transfusion therapy as the body will receive iron from the transfused RBCs, but lacks a mechanism for getting rid of any excess. However, transfusion-independent iron loading also occurs in patients with PK deficiency and is under-recognized, occurring at all ages and in patients with both mild and more severe anemia.

Transfusion-related iron loading. The association between transfusions and iron loading is similar in PK deficiency to that seen in other transfusion-dependent red cell disorders. Regular ferritin monitoring before transfusions and annual MRI assessment are indicated in patients who receive transfusions, and ongoing chelation therapy is necessary in those who receive regular transfusions.

Non-transfusion-related iron loading. Hemosiderosis also occurs frequently in individuals with PK deficiency in the absence of transfusions. Non-transfusion-related iron loading can occur at all ages and in patients with all hemoglobin levels. Although patients with both PK deficiency and hereditary hemochromatosis will have an increased risk of iron loading, ineffective erythropoiesis associated with PK deficiency is likely to be the main contributor to transfusion-independent iron loading in this hemolytic disorder. Although not confirmed, the mechanism may be similar to that seen in thalassemia intermedia, in which compensatory mechanisms to overcome chronic anemia include reduced hepcidin levels and increased gastrointestinal iron absorption.[3] Given that iron loading occurs through the gastrointestinal route, patients with PK deficiency should avoid iron supplements, including multivitamins with iron, and excessive consumption of foods high in iron.

While cardiac iron loading occurs, it is significantly less frequent than hepatic iron overload. Liver iron overload can lead to hepatic fibrosis and liver failure.

In some patients, ferritin levels significantly underestimate the degree of hepatic iron, particularly if the iron deposition is in the hepatocytes. Because iron loading is common in PK deficiency, all

patients, regardless of their transfusion status, should have their iron levels monitored at least annually with ferritin measurement. Non-transfused patients should also have an MRI assessment of hepatic and cardiac iron once a patient reaches an age where this can be done without sedation, particularly in those with ferritin levels greater than 500 ng/mL. Based on the initial findings, the frequency of subsequent MRI assessments can be determined.

Depending on the degree of iron burden, chelation therapy may be required (Table 5.3). Chelation therapy may also be needed intermittently in patients who do not receive transfusions as their MRI assessments are monitored.

Therapeutic phlebotomy can be considered, but may be less effective than chelation and may not be tolerated in significantly anemic or symptomatic patients.

Extramedullary hematopoiesis, driven by ineffective erythropoiesis, is typically found in the liver and spleen but can also occur in the paravertebral area, mediastinum and other areas. Extramedullary hematopoiesis is diagnosed by radiological studies, such as MRI, and/or a tissue biopsy. Although extramedullary hematopoiesis is not a frequent complication of PK deficiency, it is not uncommon.

Osteopenia. Patients with PK deficiency are at risk of low bone mineral density, fractures and bone pain. The risk of osteoporosis is likely to increase with age; however, this has not been well studied in patients with PK deficiency.

Regular monitoring with bone mineral density (i.e. DEXA) scans is indicated, with baseline assessment carried out in late adolescence or early adulthood. The frequency of monitoring will depend on the findings and any intervention(s). Consultation with a specialist in endocrinology may be useful in patients with low bone density, in addition to supplementation with vitamin D and calcium and exercise.

Pulmonary hypertension. Similar to other hemolytic disorders, pulmonary hypertension is a complication of PK deficiency. The etiology is not clear but is likely to be related to the ongoing hemolysis. The diagnosis can be made by echocardiogram and confirmed with right heart catheterization.

TABLE 5.3

Treatments for iron overload

Treatment (route of administration)	Monitoring*	Considerations
Deferoxamine (subcutaneous or intravenous)	• Kidney and liver function tests • CBC • Audiometry and ophthalmology examinations	Difficulty with adherence due to the requirement for daily long infusions
Deferasirox (oral)	• Kidney and liver function tests • CBC • Creatinine and creatinine clearance • Monitor for signs of gastrointestinal ulcers and/or bleeding • Audiometry and ophthalmology examinations	Often the best tolerated of the chelators. Available now as a tablet to swallow, a tablet for oral suspension or as sprinkles
Deferiprone (oral)	• Absolute neutrophil count (at baseline and weekly) • Liver function tests • Zinc levels	Risk of agranulocytosis, so mainly considered when other chelation is ineffective or with severe cardiac iron loading
Phlebotomy (Intravenous blood removal)	• CBC before phlebotomy	Safe if individual is not transfused and hemoglobin is high enough to tolerate blood removal

*In addition to regular ferritin monitoring and/or MRI. CBC, complete blood count. Note: these treatments have other potential side effects, which should be discussed in detail with the patient. Monitoring practices vary by center.

Leg ulcers have also been reported in patients with PK deficiency. The etiology is multifactorial but not well studied in this particular anemia. Skin examination should be performed at routine visits.

Special considerations in pregnancy

Pregnancy in women with PK deficiency has been associated with excellent maternal and fetal outcomes.[4] Before conception, women who have previously received a transfusion should be screened for hepatitis B and C and HIV. They should also be prescribed folic acid supplements and warned to avoid prenatal vitamins containing iron and any other iron supplement during their pregnancy. A preconception echocardiogram could be considered to evaluate cardiac function due to the chronic anemia and risk of cardiac iron overload and pulmonary hypertension.

Multidisciplinary care with a hematologist and a high-risk obstetrician is recommended, with close attention paid to fetal growth to determine transfusion frequency. Some obstetricians recommend monthly ultrasounds to assess fetal growth after 20 weeks and regular biophysical profiles by at least 32 weeks.

During pregnancy, the degree of hemolysis typically worsens and transfusion needs increase. The majority of women will be transfused during pregnancy and/or directly after the delivery, regardless of their transfusion status before pregnancy. Currently, there is not enough evidence to recommend a certain hemoglobin threshold for transfusion during pregnancy. A significant amount of iron is transferred from the mother to the fetus, which helps to balance the iron loading that could be associated with transfusions during the pregnancy.

Complications in the newborn

In the past, prenatal assessment for PK deficiency depended on the observation of symptoms during ultrasound scans. However, only severely affected cases typically appear with identifiable signs such as enlargement of the liver and spleen, or hydrops fetalis. The availability of genetic testing now allows for prenatal diagnosis of PK deficiency, with prompt diagnosis permitting more appropriate obstetric and neonatal care.

Anemia. In newborns and young infants with PK deficiency, hemoglobin levels and reticulocyte counts require frequent monitoring. However, early hemoglobin measurements are affected by the stress of birth, transition to extrauterine life and the physiological hemoglobin nadir. Therefore, it may not be possible to assess baseline hemoglobin in newborns with PK deficiency for 3–6 months, well beyond the physiological nadir. Transfusions in the first few months of life will suppress erythropoiesis and may prolong the physiological hemoglobin nadir. Extending the time between transfusions will allow erythropoietin to drive reticulocyte production and for evaluation of the true baseline hemoglobin level. Allowing an infant's hemoglobin level to drop may lead to a rise in reticulocytes followed by a rise in hemoglobin to the patient's non-suppressed baseline level.

Neonatal hyperbilirubinemia. After birth many newborns with PK deficiency will develop hemolysis with significant hyperbilirubinemia, which is treated with phototherapy and/or simple or exchange transfusions. Jaundice in newborns with PK deficiency develops due to a combination of hemolysis and immaturity of the liver. Hyperbilirubinemia can lead to kernicterus of the newborn if untreated.

Other complications. Approximately one-quarter of newborns with PK deficiency will have complications in utero or at the time of birth, including intrauterine growth retardation, hydrops fetalis, preterm birth, perinatal anemia and dermal extramedullary hematopoiesis (i.e. blueberry muffin rash). Severe hepatic disease leading to liver failure has been reported and is associated with a high rate of mortality.

Key points – complications and monitoring

- The hemolytic anemia associated with pyruvate kinase (PK) deficiency can range from mild to severe, with hemoglobin levels typically in the range of 6–12 g/dL. It can be exacerbated by hemolytic episodes and aplastic crises.
- Gallstones are a common complication of PK deficiency and the risk persists after splenectomy. Cholecystectomy should be considered at the time of splenectomy regardless of the presence of gallstones.
- Many patients with PK deficiency will have prenatal and neonatal complications including hyperbilirubinemia, prematurity, intrauterine growth retardation and perinatal anemia.
- Transfusion-independent iron loading can occur at any age and with any severity of anemia. Annual monitoring of iron is indicated in all patients with PK deficiency, regardless of transfusion status. Chelation may be intermittently prescribed.
- Other complications of PK deficiency can include low bone density, extramedullary hematopoiesis, pulmonary hypertension and leg ulcers.
- In pregnant women with PK deficiency, recommendations for management include close fetal monitoring, consideration of transfusions (because hemolysis is exacerbated) and avoidance of iron-containing supplements.

References

1. Grace RF, Bianchi P, van Beers EJ et al. The clinical spectrum of pyruvate kinase deficiency: data from the Pyruvate Kinase Natural History Study. *Blood* 2018; doi:10.1182/blood-2017-10-810796. [Epub ahead of print]

2. Tanaka KR, Paglia DE. Pyruvate kinase deficiency. *Semin Haematol* 1971;8:367–96.

3. Finkenstedt A, Bianchi P, Theurl I et al. Regulation of iron metabolism through GDF15 and hepcidin in pyruvate kinase deficiency. *Br J Haematol* 2009;144:789–93.

4. Wax JR, Pinette MG, Cartin A et al. Pyruvate kinase deficiency complicating pregnancy. *Obstet Gynecol* 2007;109:553–55.

Further reading

Glader B. Hereditary hemolytic anemias due to red blood cell enzyme disorder. In: Greer JP, Arber DA, Glader B et al, eds. *Wintrobe's Clinical Hematology*, 13th edn. Philadelphia: Lippincott Williams & Wilkins; 2014.

Grace RF, Zanella A, Neufeld EJ et al. Erythrocyte pyruvate kinase deficiency: 2015 status report. *Am J Hematol* 2015;90:825–30.

Zanella A, Fermo E, Bianchi P, Valentini G. Red cell pyruvate kinase deficiency: molecular and clinical aspects. *Br J Haematol* 2005;130:11–25.

6 Supportive treatment

At present, the main focus of care for pyruvate kinase (PK) deficiency is with supportive therapies.[1]

Transfusions

Red blood cell (RBC) transfusions are frequently used to support patients with PK deficiency, particularly during the first months of life. However, transfusion thresholds and guidelines used in other red cell disorders, such as thalassemia major or intermedia, do not necessarily apply to PK deficiency.

When to transfuse. There is no set standard with regard to transfusions for patients with PK deficiency because the degree of anemia and associated symptoms differ so much between affected individuals.[2,3] The decision to opt for transfusion therapy is therefore based on a patient's tolerance of anemia rather than on an arbitrary level of hemoglobin. As explained in Chapter 1, an increase in red cell 2,3–diphosphoglycerate results in enhanced oxygen off-loading into the tissues. Consequently, patients may tolerate moderately severe anemia with few symptoms. When patients have a transfusion, the goal nadir hemoglobin level should also be based on symptoms rather than on a level extrapolated from guidelines used in other anemias.

Many patients with PK deficiency will never need a transfusion or will only require intermittent, or unplanned, transfusions during hemolytic crises due to infections or an aplastic crisis associated with parvovirus infection. Others may remain on regular transfusion therapy until splenectomy is considered.

Hemoglobin goals. During the first years of life, hemoglobin goals are those that allow for normal growth and development, and young children are often reliant on frequent transfusions to decrease symptoms and improve growth. As individuals age through adulthood, transfusion requirements may increase despite a stable hemoglobin, as a result of increased symptoms from the anemia, perhaps related to increased daily activity.

Complications. Transfusions are associated with iron loading, and chelation therapy is necessary in patients who receive regular transfusions (see Table 5.3, page 53).

Splenectomy

The hypoxic and glucose-restricted environment of the spleen poses a problem to PK-deficient reticulocytes, which rely on oxidative phosphorylation to produce adenosine triphosphate (ATP). Impaired ATP production leads to RBC destruction in the spleen. Severely PK-deficient reticulocytes are metabolically more stable in the absence of the spleen; therefore, a paradoxical, sustained, robust reticulocytosis follows splenectomy in this disorder.

Partial splenectomy has been performed in a few patients with PK deficiency, but has not been beneficial.

The benefits of splenectomy. Before splenectomy, the reticulocyte count may be inappropriately low or mildly to moderately increased (5–15%). After splenectomy, reticulocyte counts can be as high as 50–70% and, with the extended lifespan of PK-deficient reticulocytes in the absence of the spleen, hemoglobin levels increase by a median of 1.6 g/dL.[2]

Splenectomy partially ameliorates the anemia in most patients and is beneficial in decreasing the need for transfusions in 90% of patients.[2] In patients who received regular transfusions before splenectomy, approximately 80% can discontinue transfusions altogether following surgery. However, in almost all patients, an incompletely compensated hemolytic process persists, in which mild anemia, reticulocytosis and indirect hyperbilirubinemia continue.

Poor responders. Approximately 14% of patients on regular transfusions prior to splenectomy will have a poor response and will continue to require regular transfusions.[1] In these cases, imaging to evaluate for a splenule or secondary spleen is indicated, but these are rarely found.

Patient selection. Preoperative assessment of red cell survival, splenic sequestration and/or spleen size is of no value in selecting patients for splenectomy. In part, this reflects the importance of the liver as a site of RBCl destruction. Low pre-splenectomy hemoglobin levels are associated with a poorer response to splenectomy in terms of the post-splenectomy hemoglobin rise and/or transfusion burden.

The burden of transfusions in PK deficiency varies between patients. The decision whether to have a splenectomy to improve the anemia of PK deficiency is complex and depends on both the physician's and the patient's perspective of the potential benefits and risks. Recent guidelines recommend splenectomy in patients who are reliant on regular transfusions or are severely anemic.[4] Splenectomy should also be considered in patients who receive intermittent transfusions with every infectious illness or who have symptomatic anemia.

The timing of splenectomy is based on the risk-benefit assessment. Splenectomy in young children is associated with an increased risk of post-splenectomy sepsis, while a delay in splenectomy in patients who receive regular transfusions is associated with increased iron loading.

Post-splenectomy sepsis. Splenectomy increases susceptibility to serious bacterial infections with encapsulated organisms, such as *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Neisseria meningitidis* and *Capnocytophaga canimorsus*. In the absence of a spleen, individuals are also at risk of severe babesiosis and malaria. With adequate vaccinations and prophylactic antibiotics, the absolute risk of a serious infection is very low. Nevertheless, because of these lifelong infectious risks, surgery should be avoided altogether or preferably delayed until at least 5 years of age. If splenectomy is needed at an earlier age, the benefits must be balanced against the risk of infection.

Vaccination. Since the spleen is the primary site for the production of immunoglobulin M antibodies, which are required for opsonization of encapsulated organisms, vaccination prior to splenectomy is much preferred. Vaccination schedules are frequently updated based on new information and vaccine development. Therefore, physicians should refer to an updated website rather than to articles or books for a list and schedule of vaccinations before splenectomy. A useful website is www.cdc.gov/vaccines/schedules/index.html. It is recommended that all vaccines are given at least 2 weeks before splenectomy.

Antibiotic prophylaxis. Oral antibiotics for infection prophylaxis are indicated after splenectomy. Strategies include daily antibiotic prophylaxis and empiric antibiotics administered for febrile illnesses. The ideal duration for prophylactic antibiotics is not clear, and practice varies by country. Some physicians recommend a lifetime of prophylactic antibiotics, while others recommend discontinuation after 1 year if patients live close to a medical center and agree to seek urgent medical care for all fevers.

Most physicians 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.

Once splenectomized, if an individual has a fever of 38.5°C (101.5°F) or higher, they should be seen urgently for a blood culture and complete blood count and given intravenous or intramuscular broad-spectrum antibiotics.

> Patients should be educated that a post-splenectomy fever is an emergency and medical attention should be sought even if the fever occurs at an inconvenient time or is associated with nasal congestion or other viral symptoms.

Post-splenectomy thrombosis. Many studies demonstrate an overall increased risk of thrombosis after splenectomy, including in otherwise healthy individuals. After splenectomy, the overall risk of thrombosis in PK deficiency is approximately 10%, including portal vein thrombosis, deep vein thrombosis, pulmonary embolism and central nervous system thrombosis.

Although the etiology for the increased risk is not clear, some physicians recommend taking low-dose aspirin (acetylsalicylic acid) after splenectomy, particularly in patients with marked thrombocytosis, to potentially decrease this risk.

Folic acid

With the reticulocytosis associated with PK deficiency, folic acid needs are increased. Supplemental folic acid may be needed, depending on the patient's dietary folic acid intake.

Key points – supportive treatment

- The burden of transfusions in pyruvate kinase (PK) deficiency is quite variable and depends on both patient and provider factors.
- The decision for transfusion therapy relates to the patient's tolerance of anemia rather than an arbitrary level of hemoglobin. Some patients with PK deficiency may tolerate a lower level of hemoglobin than those with other anemias due to increased red cell 2,3-diphosphoglycerate.
- Splenectomy is beneficial in increasing the hemoglobin level and decreasing the need for transfusions in most patients with PK deficiency.
- Given the potential risks associated with splenectomy, physicians should participate in shared decision making with their patients to determine whether to pursue splenectomy and at what age.
- The risk of post-splenectomy thrombosis in PK deficiency is similar (approximately 10%) to other non-malignant hematologic conditions.

References

1. Grace RF, Zanella A, Neufeld EJ et al. Erythrocyte pyruvate kinase deficiency: 2015 status report. *Am J Hematol* 2015;90:825–30.

2. Grace RF, Bianchi P, van Beers EJ et al. The clinical spectrum of pyruvate kinase deficiency: data from the Pyruvate Kinase Natural History Study. *Blood* 2018; doi:10.1182/blood-2017-10-810796. [Epub ahead of print]

3. Zanella A, Fermo E, Bianchi P et al. Pyruvate kinase deficiency: the genotype-phenotype association. *Blood Rev* 2007;21:217–31.

4. Iolascon A, Andolfo I, Barcellini W et al. Recommendations regarding splenectomy in hereditary hemolytic anemias. *Haematologica* 2017;102:1304–13.

Useful resources

ClinicalTrials.gov
Search by condition or disease for a listing of open trials in pyruvate kinase deficiency

Genetic and Rare Diseases Information Center
rarediseases.info.nih.gov/diseases/7514/pyruvate-kinase-deficiency

Genetics Home Reference
ghr.nlm.nih.gov/condition/pyruvate-kinase-deficiency

National Organization for Rare Disorders (NORD)
rarediseases.org/rare-diseases/pyruvate-kinase-deficiency

Pyruvate Kinase Deficiency Support Group
en-gb.facebook.com/pyruvatekinasedeficiencysupportgroup

Pyruvate Kinase Deficiency: Understanding and living with PKD
pyruvatekinasedeficiency.com

Agios PKR Program
www.agios.com/pipeline/pkr-program

Index

Entry