Diagnosis and epidemiology of red blood cell enzyme disorders

Richard Van Wijk

Associate professor, Laboratory For Red Blood Cell Research, Department of Clinical Chemistry and Haematology, University Medical Center Utrecht, The Netherlands

The red blood cell possess an active metabolic machinery that provides the cell with energy to pump ions against electrochemical gradients, to maintain its shape, to keep hemoglobin iron in the reduced (ferrous) form, and to maintain enzyme and hemoglobin sulfhydryl groups. The main source of metabolic energy comes from glucose. Glucose is metabolized through the glycolytic pathway and through the hexose monophosphate shunt. Glycolysis catabolizes glucose to pyruvate and lactate, which represent the end products of glucose metabolism in the erythrocyte. Adenosine diphosphate (ADP) is phosphorylated to adenosine triphosphate (ATP), and nicotinamide adenine dinucleotide (NAD)⁺ is reduced to NADH in glycolysis. 2,3-Bisphosphoglycerate, an important regulator of the oxygen affinity of hemoglobin, is generated during glycolysis by the Rapoport-Luebering shunt. The hexose monophosphate shunt oxidizes glucose-6-phosphate, reducing NADP⁺ to reduced nicotinamide adenine dinucleotide phosphate (NADPH). The red cell lacks the capacity for de novo purine synthesis but has a salvage pathway that permits synthesis of purine nucleotides from purine bases.

The red cell contains high concentrations of glutathione, which is maintained almost entirely in the reduced state by NADPH through the catalytic activity of glutathione reductase. Glutathione is synthesized from glycine, cysteine, and glutamic acid in a two-step process that requires ATP as a source of energy. Catalase and glutathione peroxidase serve to protect the red cell from oxidative damage.

Erythrocyte enzyme deficiencies may lead to hemolytic anemia(1); expression of the defect in other cell lines may lead to pathologic changes such as myopathy and neuromuscular abnormalities. Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common erythrocyte enzyme defect(2). In some populations, more than 20 percent of people may be affected by this enzyme deficiency. In common polymorphic forms, such as G6PD A- or G6PD Mediterranean, hemolysis occurs only during the stress imposed by infection or administration of oxidative drugs, and in some individuals upon ingestion

of fava beans. Patients with uncommon, functionally very severe, genetic variants of G6PD experience chronic hemolysis, a disorder designated hereditary nonspherocytic hemolytic anemia (HNSHA).(3)

Hereditary nonspherocytic hemolytic anemia also occurs as a consequence of other enzyme deficiencies, the most common of which is pyruvate kinase (PK) deficiency.(4-6) Deficiencies of glucosephosphate isomerase(6), triosephosphate isomerase(7), and pyrimidine 52-nucleotidase deficiency(8) are included among the relatively rare causes of HNSHA. In the case of some deficiencies, notably those of glutathione synthetase(9), triosephosphate isomerase(7), and phosphoglycerate kinase(10), the defect is expressed throughout the body, and neurologic and other defects may be a prominent part of the clinical syndrome.

Diagnosis is best achieved by determining red cell enzyme activity with a quantitative assay or a screening test(11). However, since the maturation of reticulocytes into erythrocytes is associated with a rapid decrease in the activity of several enzymes reticulocytosis, which is normally present, represents an important pitfall in the diagnosis of red cell enzyme deficiencies.(12)

Except for the basophilic stippling of erythrocytes which is characteristic for pyrimidine 5 nucleotidase deficiency, red cell morphology is of little or no help in differentiating red cell enzyme deficiencies from another.

Many different mutations have been defined in most of the enzyme deficiencies, in particular in PK deficiency (www.pkmutationdatabase.com). Accurate diagnosis is necessary for genetic counseling and is helpful in recommendations for treatment, since patients with some enzyme deficiencies tend to respond more favorably to splenectomy than do others. Most red cell enzyme defects are transmitted as autosomal recessive disorders, while G6PD and phosphoglycerate kinase deficiencies are X linked.

There are no exact and verified figures regarding the occurrence of red blood cell enzyme disorders, other than the number of cases reported in literature (Table 1). Basically, this is due to the lack of a certified registry. Also, like in disorders of the red cell membrane, some enzyme disorders will be difficult to identify because they are either very rare or clinically mild. This latter fact may, for instance, explain the discrepancy between the estimated number of cases affected by PK deficiency (*i.e.* 1:20,000 in the general white population) and the true number of identified cases. The European NEtwork for Rare and Congenital Anaemias (ENERCA) enabled a comparison of these numbers.(13)It was concluded that both in The Netherlands and Italy, 2 countries with a large and well-characterized database of patients with PK deficiency the true frequency was, in fact, about 10 times lower than predicted. As stated, this may either be due to a high number of patients showing a mild to very mild clinical picture or to a lack of awareness, or both. A similar situation might be applicable to haemolytic anaemia due to pyrimidine-5'-nucleotidase deficiency.

A recently conducted survey by ENERCA also brought to light that another important issue contributes to the limited knowledge on epidemiology of red cell enzyme disorders. This concerns the fact there are

Correspondence: Richard Van Wijk
E-mail: r.vanwijk@umcutrecht.nl

©Copyright R. Van Wijk, 2013
Licensee PAGEPress, Italy
Thalassemia Reports 2013; 3(s1):e33
doi:10.4081/thal.2013.s1.e33

This article is distributed under the terms of the Creative Commons Attribution Noncommercial License (by-nc 3.0) which permits any noncommercial use, distribution, and reproduction in any medium, provided the original author(s) and source are credited.

Parts of this work were presented at the "3rd Pan-European Conference on Haemoglobinopathies and Rare Anaemias", Limassol (Cyprus), 24-26 October 2012.

Table 1.

Red blood cell disorder	No. of cases
Pyruvate kinase deficiency	>500 families
Pyrimidine-5'-nucleotidase deficiency	>60 families
Triosephosphate isomerase deficiency	50 - 100 cases
Phosphofructokinase deficiency	50 - 100 cases
Phosphoglycerate kinase deficiency	40 cases
Class I glucose-6-phosphate dehydrogenase deficiency	>50 families
Glucose-6-phosphate isomerase deficiency	>50 families
Glutathione synthetase deficiency	>50 families
Hexokinase deficiency	20 cases
Adenylate kinase deficiency	12 families
Glutamate cysteine ligase deficiency	12 families
Aldolase deficiency	6 cases
Adenosine hyperactivity	3 families
Glutathione reductase deficiency	2 families

currently only a very limited number of laboratories in the EU capable of performing the necessary tests, either on the biochemical level or on the genetic level, required to diagnose red cell enzyme disorders. Whereas a considerable number of laboratories are performing diagnostic tests for detection of the two most frequently occurring red cell enzyme disorders, *i.e.* deficiencies of G6PD and PK, only very few laboratories offer the complete panel of tests for detection of the other 12 rare enzyme disorders of the red blood cell (Table 1). This fact probably strongly contributes to the relatively high amount of patients with hereditary haemolytic anaemia that remain undiagnosed. In addition, it affirms the belief among ENERCA members that the true worldwide population frequency of the rare and very rare enzyme disorders of the red blood cell may, in fact, be significantly higher than that reported in literature. This may for instance be true for a deficiency of glutathione synthetase. Further investigations into the frequency of red blood cell enzyme deficiencies are therefore warranted and should be encouraged.

References

1. van Wijk R, van Solinge WW. The energy-less red blood cell is lost: erythrocyte enzyme abnormalities of glycolysis. *Blood*. 2005;106(13):4034-42.
2. Cappellini MD, Fiorelli G. Glucose-6-phosphate dehydrogenase deficiency. *Lancet*. 2008;371(9606):64-74.
3. van Wijk R, Huizinga EG, Prins I, Kors A, Rijksen G, Bierings M, *et al.* Distinct phenotypic expression of two de novo missense mutations affecting the dimer interface of glucose-6-phosphate dehydrogenase. *Blood Cells Mol Dis*. 2004;32(1):112-7.
4. Zanella A, Fermo E, Bianchi P, Chiarelli LR, Valentini G. Pyruvate kinase deficiency: the genotype-phenotype association. *Blood Rev*. 2007;21(4):217-31.
5. Van Wijk R, Huizinga EG, Van Wesel ACW, Van Oirschot BA, Hadders MA, van Solinge WW. Fifteen novel mutations in PKLR associated with pyruvate kinase (PK) deficiency: structural implications of amino acid substitutions in PK. *Hum Mutat*. 2009;30(3):446-53.
6. Kugler W, Lakomek M. Glucose-6-phosphate isomerase deficiency. Bailliere's best practice & research Clinical haematology. 2000;13(1):89-101. Epub 2000/08/05.
7. Orosz F, Oláh J, Ovádi J. Triosephosphate isomerase deficiency: New insights into an enigmatic disease. *Biochim Biophys Acta*. 2009;1792(12):1168-74. Epub 2009/09/30.
8. Zanella A, Bianchi P, Fermo E, Valentini G. Hereditary pyrimidine 5'-nucleotidase deficiency: from genetics to clinical manifestations. *Br J Haematol*. 2006;133(2):113-23. Epub 2006/04/14.
9. Njålsson R, Ristoff E, Carlsson K, Winkler A, Larsson A, Norgren S. Genotype, enzyme activity, glutathione level, and clinical phenotype in patients with glutathione synthetase deficiency. *Hum Genet*. 2005;116(5):384-9.
10. Beutler E. PGK deficiency. *Br J Haematol*. 2007;136(1):3-11. Epub 2007/01/16.
11. Beutler E. Red cell metabolism. A manual of biochemical methods. 3rd ed. Beutler E, editor. Orlando: Grune & Stratton; 1984. 188 p.
12. van Wijk R, van Wesel ACW, Rijksen G, van Solinge WW. *Ex vivo* analysis of PKLR mutations that affect correct processing of PKLR mRNA causing pyruvate kinase deficiency. *Haematologica*. 2006;91(s1):8.
13. ENERCA WhiteBook for the creation of a European Reference Network of Centres of Expertise on Rare anaemias (in press).