Study of (increased) expression of red blood cell pyruvate kinase antigen and ineffective erythropoiesis in patients with red blood cell pyruvate kinase deficiency.

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Primary Objective: The here described study aims to strengthen the recently obtained results regarding increased expression of PK antigen in patients with PK-deficiency. This will be achieved by increasing the number of investigated patients and...

Ethical review	Approved WMO
Status	Recruitment stopped
Health condition type	Blood and lymphatic system disorders congenital
Study type	Observational invasive

Summary

ID

NL-OMON35096

Source
ToetsingOnline

Brief title
PKR-Ag expression in PKD patients.

Condition

- Blood and lymphatic system disorders congenital

Synonym
anemia, red blood cell disease

Research involving
Human

Sponsors and support

Primary sponsor: Universitair Medisch Centrum Utrecht
Source(s) of monetary or material Support: Ministerie van OC&W

Intervention

Keyword: erythropoiesis, hemolytic anemia, pyruvate kinase, red blood cells

Outcome measures

Primary outcome

Main study parameter: PK antigen levels in patients with PK-deficiency.

Red blood cell concentrates will be generated from 6 ml peripheral blood samples (EDTA) from PK-deficient patients and controls according to well-established methods. Subsequently, PK antigen levels will be determined by an ELISA method that has been recently developed in our laboratory (Van Wijk et al, 2009). Levels will be compared with normal control samples and correlated to the clinical phenotype and the genotype of patients.

Van Wijk, R., Huizinga, E.G., Van Wesel, A.C.W., Van Oirschot, B.A., Hadders, M.A. & van Solinge, W.W. (2009) Fifteen novel mutations in PKLR associated with pyruvate kinase (PK) deficiency: structural implications of amino acid substitutions in PK. Hum Mutat, 30, 446-453.

Secondary outcome

Secondary study parameters:

(1) Capability of human PK-deficient hematopoietic stem cells to differentiate into erythroid cells, and pro-apoptotic gene expression in PK-deficient erythroid cells.

The peripheral blood mononuclear cells fraction will be obtained from 20 ml peripheral blood samples from PK-deficient patients and controls by density gradient centrifugation. Subsequently, these cells will be cultured in vitro in the presence of growth factors, including erythropoietin, for approximately 2 weeks. Erythroid colonies will be counted and characterized. Numbers and characteristics will be compared to normal control samples.
After counting and characterization, erythroid colonies will be harvested and total RNA will be isolated. This RNA will be used to study expression of pro-apoptotic genes like, for example, BAD, BNIP3, and BNIP3l by quantitative RT-PCR methods. Gene expression levels will be calculated relative to normal control expression levels.

(2) PK-deficient red blood cell characteristics (red blood cell counts), iron status, ane erythropoietin levels.
In order to interpret results and to classify PK-deficient patients according to their fenotype, general red blood cell parameters (e.g. hemoglobin, reticulocyte counts), the patient's iron status as well as erythropoietin levels should be taken into account. For this, 6 ml lithium-heparine (erythropoietin levels) and 3 ml EDTA peripheral blood samples will be collected from PK-deficient patients. Laboratory tests will be performed by the Central Diagnostic Laboratory of the Department of Clinical Chemistry and Haematology. Results will be compared with the reference values of the laboratory.

Study description

Background summary

The mature red blood cell is completely dependent on glycolysis for it*s energy supply. Pyruvate Kinase (PK) is a key enzyme of glycolysis. Deficiency of this protein is the most frequently occuring glycolytic enzyme disorder and an important cause of hereditary non-spherocytic haemolytic anaemia (van Wijk and van Solinge 2005). Clinical symptoms of PK deficiency are usually limited to patients who are compound heterozygous or homozygous for a mutation in PKLR. The phenotypic expression is highly variable (Zanella, et al 2007), and patients with identical genotypes may show a diverse clinical picture (van Wijk and van Solinge 2006). Also, there is no relationship between the residual enzymatic activity and the severity of haemolysis. Hence, in PK deficiency the genotype-to-phenotype correlation is poor. Metabolically, deficiency of PK results in decreased enzymatic activity and, consequently, ATP depletion and increased levels of 2,3-diphosphoglycerate. Eventually this leads to red cell destruction but the precise mechanisms that lead to a shortened lifespan of the PK-deficient red blood cell are as-yet unknown (Valentine and Paglia 1980).
The number of haematopoietic progenitors, including CFU-GM, BFU-E and CFU-GEMM, in the spleen of PK-deficient patients are much higher than in spleens of control subjects (Aizawa, et al 2003). This indicates that increased extramedullary haematopoiesis takes place in PK-deficient patients. Of particular interest is the fact that cells undergoing apoptosis have been detected in splenic red pulp of a PK deficient patient whereas there was an absence of detectable apoptotic cells in control samples (Aizawa, et al 2003). This strongly suggests that PK activity is required for the maturation of erythroid progenitors by preventing these cells from apoptosis. Also in mice it has been shown that red blood cell PK deficiency is associated with ineffective erythropoiesis (Aizawa, et al 2005). In addition, further studies have revealed that over-expression of recombinant wild-type PK in a PK-deficient cell murine cell line is able to downregulate expression of pro-apoptotic genes, such as Bad, Bnip3, and Bnip3l (Aisaki, et al 2007).
Recently we have recently shown that loss of PK enzymatic activity is not accompanied by a quantitative reduction in the amount of PK but, rather, is associated with (strongly) increased levels of PK antigen in about half of the patients examined (Van Wijk, et al 2009). This is an intriguing observation in light of the fact that in many of these patients the underlying mutation in PKLR predicts instability of the PK monomer and/or tetramer. The results suggest that the metabolic disturbances induced by PK deficiency lead to an increased expression of PKLR during erythroid differentiation and maturation. Consequently, these observations led us to hypothesize that increased expression of PK may be compensatory, i.e. to correct for the loss of enzymatic activity, but may also serve to protect the PK-deficient erythroid progenitor cell from apoptosis by downregulating pro-apoptopic gene expression. PK-antigen

levels may thus be an important determinant of the ultimate clinical phenotype of patients with PK deficiency and, as such, important for a better understanding of the complex genotype-to-phenotype correlation in this disease.

Aisaki, K., Aizawa, S., Fujii, H., Kanno, J. & Kanno, H. (2007) Glycolytic inhibition by mutation of pyruvate kinase gene increases oxidative stress and causes apoptosis of a pyruvate kinase deficient cell line. Exp Hematol, 35, 1190-1200.
Aizawa, S., Harada, T., Kanbe, E., Tsuboi, I., Aisaki, K., Fujii, H. & Kanno, H. (2005) Ineffective erythropoiesis in mutant mice with deficient pyruvate kinase activity. Exp Hematol, 33, 1292-1298.
Aizawa, S., Kohdera, U., Hiramoto, M., Kawakami, Y., Aisaki, K.-I., Kobayashi, Y., Miwa, M., Fujii, H. & Kanno, H. (2003) Ineffective erythropoiesis in the spleen of a patient with pyruvate kinase deficiency. Am J Hematol, 74, 68-72.
Valentine, W.N. & Paglia, D.E. (1980) The primary cause of hemolysis in enzymopathies of anaerobic glycolysis: a viewpoint. Blood Cells, 6, 819-829.
Van Wijk, R., Huizinga, E.G., Van Wesel, A.C.W., Van Oirschot, B.A., Hadders, M.A. & van Solinge, W.W. (2009) Fifteen novel mutations in PKLR associated with pyruvate kinase (PK) deficiency: structural implications of amino acid substitutions in PK. Hum Mutat, 30, 446-453.
van Wijk, R. & van Solinge, W.W. (2005) The energy-less red blood cell is lost: erythrocyte enzyme abnormalities of glycolysis. Blood, 106, 4034-4042.
van Wijk, R. & van Solinge, W.W. (2006) Pyruvate kinase deficiency: genotype to phenotype. Hematology (EHA Educ Program), 2, 55-62.
Zanella, A., Fermo, E., Bianchi, P., Chiarelli, L.R. & Valentini, G. (2007) Pyruvate kinase deficiency: the genotype-phenotype association. Blood Rev, 21, 217-231.

Study objective

Primary Objective: The here described study aims to strengthen the recently obtained results regarding increased expression of PK antigen in patients with PK-deficiency. This will be achieved by increasing the number of investigated patients and control subjects. The level of PK antigen will be correlated to the clinical phenotype and the genotype.
Secondary Objective(s): This study further aims to investigate the capability of human PK-deficient haematopoietic stem cells to differentiate into erythroid cells, and to study pro-apoptotic gene expression in PK-deficient erythroid cells.

Study design

This study will be conducted as an observationel case control study. Blood samples will be collected from 25 PK-deficient patients, and 25 control subjects. These samples will be used to investigate:
1. the level of PK-antigen in mature red blood cells

2. the in-vitro ability of haematopoietic stem cells to form BFU-E*s and CFU-E*s
3. the expression of pro-apoptotic gene expression (e.g. BAD, BNIP3, BNIP3l) in erythroid progenitor cells.

Study burden and risks

Patients and control subjects will undergo a single drawing of 35 mL of blood by
venapunction. In case of children under the age of 12 years, this amount will be limited to 20 mL. To limit any discomfort, the collection of blood for this study will be combined with scheduled routine venapunctions for control visits.

Contacts

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Trial sites

Listed location countries

Netherlands

Eligibility criteria

Age
Adolescents (12-15 years)
Adolescents (16-17 years)
Adults (18-64 years)

Children (2-11 years)
Elderly (65 years and older)

Inclusion criteria

genetically confirmed diagnosis of pyruvate kinase deficiency

Exclusion criteria

Patients may not have received blood transfusions within 3 months prior to blood collection.

Study design

Design

Study type:	Observational invasive
Intervention model:	Other
Allocation:	Non-randomized controlled trial
Masking:	Open (masking not used)
Control:	Active
Primary purpose:	Other

Recruitment

NL

Recruitment status:	Recruitment stopped
Start date (anticipated):	17-08-2010
Enrollment:	50
Type:	Actual

Ethics review

Approved WMO

Date:	26-07-2010
Application type:	First submission
Review commission:	METC Universitair Medisch Centrum Utrecht (Utrecht)

Study registrations

Followed up by the following (possibly more current) registration

No registrations found.

Other (possibly less up-to-date) registrations in this register

No registrations found.

In other registers

Register	ID
CCMO	NL31362.041.10