Al-Samkari Hanny (Orcid ID: 0000-0001-6175-1383)
Chonat Satheesh (Orcid ID: 0000-0002-5909-0800)
Kwiatkowski Janet (Orcid ID: 0000-0001-7103-3406)
Verhovsek Madeleine (Orcid ID: 0000-0002-5022-9765)
Sheth Sujit (Orcid ID: 0000-0002-7640-1244)
Addonizio Kathryn (Orcid ID: 0000-0003-1574-5665)
Grace Rachael (Orcid ID: 0000-0001-7302-0449)

Characterization of the Severe Phenotype of Pyruvate Kinase Deficiency

Hanny Al-Samkari, MD[1], Eduard J. van Beers, MD, PhD[2], D. Holmes Morton, MD[3], Wilma Barcellini, MD[4], Stefan W. Eber, MD[5], Bertil Glader, MD, PhD[6], Hassan M. Yaish, MD[7], Satheesh Chonat, MD[8], Kevin H. M. Kuo, MD[9], Nina Kollmar, MD[10], Jenny M. Despotovic, DO[11] , Dagmar Pospíšilová , MD, PhD[12], Christine M. Knoll, MD[13], Janet L. Kwiatkowski, MD[14], Yves D. Pastore, MD[15], Alexis A. Thompson, MD[16], Marcin W. Wlodarski, MD[17,18], Yaddanapudi Ravindranath, MBBS[19], Jennifer A. Rothman, MD[20] , Heng Wang, MD, PhD[21], Susanne Holzhauer, MD[22], Vicky R. Breakey, MD[23], Madeleine M. Verhovsek, MD[23], Joachim Kunz, MD[24], Sujit Sheth, MD[25], Mukta Sharma, MD[26], Melissa J. Rose, DO[27], Heather A. Bradeen, MD[28], Melissa N. McNaull, MD[29], Kathryn Addonizio, BA[30], Hasan Al-Sayegh, MBChB, MPH[30], Wendy B. London, PhD[30], Rachael F. Grace, MD[30]

[1]Division of Hematology, Massachusetts General Hospital, Harvard Medical School, Boston, MA; [2]Universitair Medisch Centrum Utrecht, Utrecht; [3]Central Pennsylvania Clinic for Special Children & Adults, Belleville, PA; Lancaster General Hospital, Lancaster, PA; [4]Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Milan, Italy; [5]Schwerpunktpraxis für Pädiatrische Hämatologie- Onkologieand Children's Hospital, Technical University, Munich, Germany; [6]Lucile Packard Children's Hospital, Stanford University, Palo Alto, CA; [7]Primary Children's Hospital, University of Utah, Salt Lake City, UT; [8]Emory University School of Medicine , Aflac Cancer and Blood Disorders Center, Children's Healthcare of Atlanta, GA, Atlanta, GA; [9]University of Toronto, University Health Network, Ontario, Canada; [10]Klinikum Kassel GmbH, Kassel, Germany; [11]Texas Children's Hematology Center, Baylor College of Medicine, Houston, TX; [12]Fakultni nemocnice Olomouc, Czech Republic; [13]Phoenix Children's Hospital, Phoenix, AZ; [14]Children's Hospital of Philadelphia and Perelman School of Medicine of the University of Pennsylvania, Philadelphia, PA; [15]CHU Sainte-Justine, Montreal, Canada; [16]Ann & Robert H. Lurie Children's Hospital of Chicago, Chicago, IL; [17]St. Jude Children's Research Hospital, Memphis, TN; [18]University of Freiburg, Freiburg, Germany; [19] Children's Hospital of Michigan, Wayne State University School of Medicine, Detroit, MI; [20]Duke University Medical Center, Durham, NC; [21]DDC Clinic for Special Needs Children, Middlefield, OH; [22]Charité University Medicine, Berlin, Germany; [23]McMaster University, Hamilton, ON, Canada; [24]Zentrum für Kinder-und Jugendmedizin, Heidelberg, Germany; [25]Weill Cornell Medical

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/ajh.25926

College, New York Presbyterian Hospital, New York, NY; [26]Children's Mercy, University of Missouri Kansas City School of Medicine, Kansas City, MO; [27]Nationwide Children's Hospital, The Ohio State University College of Medicine, Columbus, OH; [28]The University of Vermont Children's Hospital, Burlington, VT; [29]University of Mississippi Medical Center, Jackson, MS; [30]Dana-Farber Boston Children's Cancer and Blood Disorder Center, Harvard Medical School, Boston, MA

Address correspondence to:
Hanny Al-Samkari, M.D.
Division of Hematology
Massachusetts General Hospital
Zero Emerson Place Suite 118 Office 112
Boston, MA 02114
hal-samkari@mgh.harvard.edu
Phone: 617-643-6214
Fax: 617-643-8840

Running title: Characterization of Severe PK Deficiency
Word count: 1102
Figures: 0
Tables: 2
References: 7
Key words: Pyruvate kinase, PK deficiency, severe, transfusion, transfusion-dependent, hemolysis

TO THE EDITOR:

Pyruvate kinase (PK) deficiency is the most common cause of hereditary non-spherocytic hemolytic anemia and is characterized by considerable genotypic heterogeneity with over 350 documented pathogenic mutations in the *PKLR* gene.[1,2] Clinical manifestations range from a mild, asymptomatic well-compensated anemia to a severe transfusion-dependent hemolytic anemia from birth.[3,4] Other complications of PK deficiency include iron overload, pulmonary hypertension, endocrinopathies, liver failure, biliary disease, and extramedullary hematopoiesis, among others.[3,4] Splenectomy, a common supportive treatment, may partially ameliorate the anemia and reduce transfusion requirements.[5] Hemoglobin concentrations correlate poorly with symptoms in PK deficiency;[6] therefore, transfusion requirements are typically used to classify disease severity, with those who are regularly transfused (often labeled "transfusion-dependent") despite splenectomy assumed to be the most severely-affected subgroup. Our understanding of the clinical characteristics of this most severe subgroup is quite limited. Therefore, in this study, we aimed to describe the differences in clinical characteristics and disease complications between regularly transfused splenectomized patients with PK deficiency and those who were also splenectomized but did not require regular transfusions. As is observed in other hereditary hemolytic anemias, we hypothesized that patients with pyruvate kinase deficiency requiring regular transfusions would have higher rates of disease complications.

Using data collected from the Pyruvate Kinase Deficiency Natural History Study (PKD NHS),[3] a prospective, international 30-site observational study, we aimed to compare demographics, complications, and laboratory results between two groups of patients with PK deficiency, defined by their clinical severity. All participants in the PKD NHS had molecularly confirmed PK deficiency and only splenectomized patients were included in this analysis. Regular transfusions were defined as ≥6 discrete red cell transfusion episodes per year. Transfusion frequency was observed over a 3-year period (divided into three 1-year periods) post-splenectomy. Patients were categorized into two groups based on transfusion frequency and splenectomy status. The most severe PK deficiency phenotype group was defined as those who received regular transfusions post-splenectomy. The comparison PK deficiency group did not receive regular transfusions post-splenectomy. Phenotype stability over the 3-year period was also assessed. Continuous parameters were compared between the two groups using the Wilcoxon rank-sum test and binary parameters were compared using the Fisher's exact text. Different genotype classifications between the two groups were compared using the Cochran-Armitage test.

Of the 255 patients enrolled in the PKD NHS, 154 splenectomized patients were included in this analysis (30 patients in the most severe PK deficiency phenotype group and 124 patients in the comparison PK deficiency group). Patients in the most severe PK deficiency phenotype group were followed in 21 of the 30 participating centers.

Results of the analysis comparing the two groups are described in Table 1. Given our definition of disease-severity, the most severely affected patients were more likely to have iron overload (93% vs. 51%, *P*<0.0001), have received chelation therapy (90% vs. 42%, *P*<0.0001), and have had more lifetime transfusions (median: 77 versus 15, *P*<0.0001) than the comparison PK deficiency group. The most severe patients were more likely to be female (77% versus 51%, *P*=0.013) and older at the time of splenectomy (median age: 5 versus 3.6, *P*=0.011). Rates of other PK deficiency complications including pulmonary hypertension, extramedullary hematopoiesis, liver cirrhosis, endocrinopathy, and bone fracture appeared similar between the two groups. Laboratory values, including hemoglobin, total bilirubin, normalized PK enzyme activity, and median absolute reticulocyte count appeared similar between the two groups. In a second analysis comparing the two groups but with exclusion of the Amish population (N=52), findings were unchanged except that the age of splenectomy was no longer significantly different (median: 5.1 vs. 4.9 years, *P*=0.96) between the most severe versus comparator PK deficiency group. The underlying genetic mutation patterns (missense mutations vs. non-missense, such as frameshift variants or deletions) also appeared similar between the groups. Phenotype over time was fairly stable for patients who met the criteria for the comparator group at the time of enrollment, but variable for patients who met the criteria for most severe at enrollment, year 1, or year 2 (Table 2). Many of the latter patients alternated severity level from year-to-year; just six (27%) of

the 22 patients who were most severe at any time point met the definition of most severe at all three time points.

In summary, splenectomized patients with PK deficiency who are not regularly transfused appear to have similar rates of PK deficiency-associated complications (except for iron overload) and similar relevant laboratory values and genotypes when compared to those who are regularly transfused. If severe hemolysis results in higher rates of PK deficiency complications and hemolysis severity drives transfusion frequency, the findings of this study are counterintuitive, in that iron overload (and associated chelation therapy, an expected complication of transfusions), was the only PK deficiency complication more common in the most severe phenotype group. The similarity observed between the most severe phenotype patients and comparison PK deficiency patients could result from a protective effect of transfusion, as has been described in thalassemia intermedia.[7] It could also suggest transfusion-dependence does not truly reflect disease severity, but is reflective of varying provider practices and/or patient symptoms. The latter hypothesis is supported by the fact that many patients classified as severe at baseline no longer met criteria for this classification one and two years later, demonstrating that transfusion requirements in patients with PK deficiency fluctuate significantly over time. Transfusion-dependence, therefore, does not necessarily imply a worse clinical outcome in PK deficiency. Furthermore, there are no transfusion guidelines for PK deficiency to help determine a universal transfusion

threshold. Early in life, transfusion requirements often decrease with age likely due to fewer infection-associated hemolytic episodes, timing of splenectomy, and age-differences in tolerance of anemia. The opposite is sometimes seen later in life, with transfusion requirements increasing during middle-age, possibly due to physiologic age-related decline in cardiopulmonary fitness. Physiologic determinants of severity of disease in PK deficiency likely significantly relate to intrinsic red cell factors, such as PK protein levels[8] or non-PKLR red cell gene variants, of which transfusion requirements may be a poor surrogate.

Our analysis is limited by the lack of a standard threshold in patients with PK deficiency for the number of transfusions per year that meet criteria for regularly transfused. Use of a stricter definition (≥7 transfusions per year) would have significantly diminished the small sample size making comparisons between the two groups difficult and likely diminished the relevance of the analysis as it would be defining a rather niche group in an already rare disease. Furthermore, the indication and goals for initiating (and discontinuing) regular transfusions, for example to treat a complication or improve subjective symptoms, were not collected.

In conclusion, the most severely affected patients with PK deficiency defined as a requirement for regular RBC transfusions following splenectomy have a similarly high rate of disease complications and laboratory abnormalities as splenectomized patients not requiring regular transfusions, with the exception of higher rates of iron overload.

Transfusion requirements seem to fluctuate considerably over time and are not a valid marker of disease severity in PK deficiency. A prospective study of a universal transfusion protocol may be useful in understanding the role of transfusions in the management of patients with PK deficiency.

Acknowledgements: H. Al-Samkari is the recipient of the National Hemophilia Foundation-Shire Clinical Fellowship Award, the Harvard KL2/Catalyst Medical Research Investigator Training Award, and the American Society of Hematology Scholar Award.

Authorship Contributions: H. Al-Samkari wrote the first draft of the manuscript and contributed to data analysis, creation of tables and figures, critical revision of the manuscript, and final approval; H. Al-Sayegh contributed to data analysis, critical revision of the manuscript, and final approval; W. London contributed to data analysis, critical revision of the manuscript, and final approval; R. Grace contributed to study design, data collection, data analysis, creation of tables and figures, critical revision of the manuscript, and final approval; and the following authors contributed to data collection, critical revision of the manuscript, and final approval: E. van Beers, D. Morton, W. Barcellini, S. Eber, B. Glader, H. Yaish, S. Chonat, K. Kuo, N. Kollmar, J. Despotovic, D. Pospíšilová, C. Knoll, J. Kwiatkowski, Y. Pastore, A. Thompson, M. Wlodarski, Y. Ravindranath, J. Rothman, H. Wang, S. Holzhauer, V. Breakey, M. Verhovsek, J. Kunz, S. Sheth, M. Sharma, M. Rose, H. Bradeen, M. McNaull, K. Addonizio.

Disclosures: Al-Samkari: Consultancy (Agios, Dova, Moderna), Research funding (Agios, Dova, Amgen). Van Beers: Consultancy and Research Funding (Agios). Barcellini: Consultancy (Agios, Alexion, Apellis, Biocryst, Bioverativ, Incyte, Momenta, Novartis). Eber: Consultancy (Agios). Kollmar: None. Despotovic: None. Pospíšilová: None. Knoll: None. Thompson: Consultancy (Bluebirdbio, Celgene, Novartis), Research Funding (Bluebirdbio, Celgene, Novartis, Baxalta). Wlodarski: None. Glader: Scientific Advisor and Consultancy (Agios), Research Funding (Agios). Morton: None. Chonat: Advisory board (Alexion, Agios). Kuo: Honoraria (Alexion, Novartis); Consultancy

(Agios, Alexion, Bluebirdbio, Celgene, Novartis, Pfizer); Data Safety Monitoring Board Chair (Bioverativ). Holzhauer: Consultancy (Pfizer, Agios, Novartis), Research Funding (Pfizer). Verhovsek: None. Kunz: Consultancy (Novartis, Bluebird Bio). Sheth: None. Rose: None. Bradeen: None. McNaull: None. Rothman: Consultancy and Research Funding (Agios). Kwiatkowski: Consultancy (Celgene, Bluebirdbio, Imara, Agios). Research Funding (Apopharma, Novartis, Terumo, Bluebirdbio). London: Consultancy (ArQule, Jubliant Draximage) and Research Funding (Agios, Bluebird Bio, Rocket Pharma). Grace: Consultancy (Agios, Dova), and Research Funding (Agios, Novartis, Pfizer). The remaining authors have no conflicts of interest.

REFERENCES

1. Warang P, Kedar P, Ghosh K, Colah R. Molecular and clinical heterogeneity in pyruvate kinase deficiency in India. *Blood Cells Mol Dis.* 2013;51(3):133-137.
2. Pissard S, Max-Audit I, Skopinski L, et al. Pyruvate kinase deficiency in France: a 3-year study reveals 27 new mutations. *Br J Haematol.* 2006;133(6):683-689.
3. Grace RF, Bianchi P, van Beers EJ, et al. Clinical spectrum of pyruvate kinase deficiency: data from the Pyruvate Kinase Deficiency Natural History Study. *Blood.* 2018;131(20):2183-2192.
4. Al-Samkari H, van Beers EJ, Kuo KHM, et al. The variable manifestations of disease in pyruvate kinase deficiency and their management. *Haematologica.* 2020.
5. Necheles TF, Finkel HE, Sheehan RG, Allen DM. Red cell pyruvate kinase deficiency. The effect of splenectomy. *Arch Intern Med.* 1966;118(1):75-78.
6. Oski FA. Clinical consequences of enzyme deficiencies in the erythrocyte. *Annals of clinical and laboratory science.* 1971;1(2):177-183.
7. Vichinsky E. Non-transfusion-dependent thalassemia and thalassemia intermedia: epidemiology, complications, and management. *Curr Med Res Opin.* 2016;32(1):191-204.
8. Al-Samkari H, Addonizio K, Glader B, et al. The pyruvate kinase (PK) to hexokinase enzyme activity ratio and erythrocyte PK protein level in the diagnosis and phenotype of PK deficiency. *Br J Haematol.* 2020.

TABLES

Table 1. Demographics, clinical characteristics, and laboratory characteristics of the most severe phenotype PK deficiency group vs. the comparison PK deficiency group (n=154 splenectomized patients with PK deficiency).

	Most severe PK deficiency phenotype: Splenectomized patients receiving regular transfusions, N=30		**Comparison PK deficiency group: Splenectomized patients not receiving regular transfusions, N=124**		***P* value****
	N*	Median (range) or n (%)	N*	Median (range) or n (%)	
Age at diagnosis (years)	29	0.7 (0-47.6)	117	0.2 (0-42.3)	0.2
Age at enrollment (years)	30	26.2 (1.4-58.5)	124	22.7 (0.3-60.4)	0.95
Gender **Female** **Male**	30	 23 (77%) 7 (23%)	122	 63 (51%) 61 (49%)	0.013
Amish	30	1 (3%)	124	51 (41%)	<0.0001
Splenectomy	30	30 (100%)	124	124 (100%)	-
Age at splenectomy (years)	30	5 (1.6-25.8)	124	3.6 (0.4-37.8)	0.011
Median hemoglobin post-splenectomy (g/dL)[@]	29	8.6 (6.3-11)	122	8.8 (6.5-12.3)	0.3
Mean total bilirubin at enrollment (mg/dL)[@]	24	3.5 (1.3-11)	104	3.9 (1-17.6)	0.95
Median lactate dehydrogenase at enrollment (U/L)	17	283.5 (142-1043)	57	215 (144-1007)	0.2
Median absolute reticulocyte count (x 10^6 cells/μL)[@]	17	1.2 (0-681)	56	0.8 (0.1-1274.6)	0.09
Pulmonary hypertension	30	3 (10%)	122	5 (4%)	0.2
Extramedullary hematopoiesis	29	3 (10%)	121	21 (17%)	0.6
Liver cirrhosis	30	2 (7%)	122	5 (4%)	0.6
Endocrinopathy **Growth Hormone Deficiency** **Hypoparathyroidism** **Hypogonadal Hypogonadism** **Thyroid Disease** **Diabetes**	 30 30 30 30 30	 1 (3%) 2 (7%) 1 (3%) 5 (17%) 2 (7%)	 124 124 124 122 124	 4 (3%) 2 (2%) 1 (1%) 7 (6%) 2 (2%)	 1 0.2 0.4 0.06 0.2
Bone fracture (any)	30	7 (23%)	124	27 (22%)	0.8

History of iron chelation	30	27 (90%)	124	52 (42%)	<0.0001
Iron overload (defined by maximum ferritin >1000 ng/ml and/or iron chelation)	30	28 (93%)	108	55 (51%)	<0.0001
Total number of lifetime transfusions	29	77 (10-544)	121	15 (0-492)	<0.0001
Normalized PK enzyme activity* (%) at enrollment**	15	-50 (-123.3-117.6)	31	-48.5 (-201.6-56.6)	0.5
Genotype groups**	29		72		0.7
M/M		14 (48%)		37 (51%)	
M/NM		8 (28%)		21 (29%)	
NM/NM		7 (24%)		14 (19%)	

*Sample sizes are those with known data for the given characteristic.
@ Pre-transfusion values
M: missense, NM: non-missense
**Using Wilcoxon rank-sum test for continuous parameters, Fisher's exact test for binary parameters, and Cochran-Armitage Trend Test for the genotype groups comparison.
***The normalized PK activity was calculated as: $[(PK_{obs} - PK_{LL}) \times 100] / (PK_{UL} - PK_{LL})$ where PK_{obs} is the observed PK enzyme value, and PK_{LL} and PK_{UL} are the lower and upper limits of the reference range, respectively.
****Analysis excluded Amish patients.

Table 2. Severity status of splenectomized patients with PK deficiency at three time-points (enrollment, first follow-up year, second follow-up year), for patients with known severity status at all three time-points (N=116).

Subgroup	Most severe at enrollment	Most severe at Year 1	Most severe at Year 2	n (%)	Comment
Comparator PK deficiency group	No	No	No	94 (81%)	Not most severe at all time-points
Most severe PK deficiency group	No	No	Yes	5 (4%)	
	No	Yes	No	2 (2%)	
	No	Yes	Yes	3 (3%)	
	Yes	No	No	3 (3%)	
	Yes	Yes	No	3 (3%)	
	Yes	Yes	Yes	6 (5%)	Most severe at all time-points