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Prevalence and management of iron overload in pyruvate kinase deficiency: report from the Pyruvate Kinase Deficiency Natural History Study

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Title: Prevalence and management of iron overload in pyruvate kinase deficiency: report from the Pyruvate Kinase Deficiency Natural History Study

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Pyruvate kinase (PK) deficiency is the most common red cell glycolytic enzyme defect causing hereditary non-spherocytic hemolytic anemia. Current treatments are mainly supportive and include red cell transfusions and splenectomy.¹¹ Regular red cell transfusions are known to result in iron overload; however, the prevalence and spectrum of transfusion-independent iron overload in the overall PK deficient population has not been well defined. This analysis describes the prevalence and clinical characteristics of iron overload in patients enrolled in the PK Deficiency Natural History Study (NHS) with a focus on those patients who are not regularly transfused.²

The PK deficiency NHS protocol (NCT02053480) was approved by each site's Institutional Review Board and/or Ethics Committee, and study procedures were in accordance with the Helsinki declaration. In Lancaster, Pennsylvania, in which all enrolled patients are Amish, additional laboratory and radiologic data were collected under a site-specific IRB-approved protocol. All patients gave informed consent. The NHS enrolled 278 patients with PK deficiency from June 2014 through April 2017 at 31 centers in 6 countries. A detailed description of the cohort is published elsewhere.² Twenty-four patients were excluded due to the inability to confirm two pathogenic *PKLR* mutations. Patients less than one year old at enrollment were also excluded (n=12) from this analysis, because ferritin is less reliably related to iron overload in this youngest age group, leaving 242 participants reported herein. Patients were defined as regularly transfused if they had received ≥ 6 transfusions in the 12 months prior to enrollment. At enrollment, 82% (198/242) of patients were not receiving regular transfusions; 38% (53/138) of these patients had iron overload as defined by ferritin. The patients with iron overload were older, more anemic, and more often splenectomized, and had a higher median total bilirubin (Table 1).

Baseline and retrospective clinically available data were used. Serum ferritin levels and quantitative (T2*) liver and heart MRIs were used to assess iron overload. Patients were considered to have iron overload if (i) their highest ferritin was over 1000 ng/ml, or (ii) they received chelation therapy in the 12 months prior to enrollment, or (iii) their highest liver iron concentration (LIC) was >3 mg/g dry weight liver (DW) on T2* MRI, OR (iv) they had cardiac iron overload as defined by a cardiac T2* ≤ 20 ms at any time in their history. The definition of iron overload as an LIC >3 mg/g DW was based on guidelines for β -thalassemia.^{3,4}

Baseline characteristics and results are listed in Table 1. Of the 242 patients, 175 (72%) had ferritin levels measured within the prior 12 months. The median ferritin was 583 ng/ml (range: 17-5630 ng/ml). The overall prevalence of iron overload as defined by ferritin or chelation was 45% (82/181). Patients without ferritin monitoring had fewer transfusions (1% vs 25% regularly transfused, $p < 0.0001$) and a higher hemoglobin (Hb) level (median Hb 9.6 vs. 8.8 g/dl, $p = 0.01$, Supplemental Table 1).

An MRI for liver iron assessment was conducted in 65 (27%) patients in the 12 months prior to enrollment; 47 (72%) were from the Amish cohort and obtained per protocol. Of the patients who were not receiving regular transfusions that had MRI or chelation data available, 82% (67/82) had iron overload as defined by MRI or chelation (Table 1). Of those patients who had never been transfused and had MRI or chelation data available, 6 of 7 patients met criteria for iron overload (6/7 splenectomized).

MRI for cardiac iron assessment was available for 75 (31%) patients. Five patients (7%) had cardiac iron overload; only one of these patients had LIC measured (5 mg Fe/g DW). These patients' ages ranged from 3-34 years with a median number of lifetime transfusions of 39 (range 10-90).

There were 68 patients ages 1-<10 years old. Of those who were not regularly transfused at enrollment but had received occasional transfusions, 82% (9/11) had iron overload as defined by MRI. The median number of lifetime transfusions in this group was 22.5 (range: 11-85). Of those <10 years old who had never been transfused (n=9), MRI was available for only one patient, which confirmed iron overload.

Forty-five patients had paired ferritin and LIC measurements available (Figure 1). Using a ferritin cut off of 1000 ng/ml, the sensitivity to predict LIC >3 mg/g DW was 53% and the specificity was 100%. At a ferritin cut-off of 500 ng/ml, the sensitivity for LIC >3 mg/g DW was 90% and the specificity was 67%.

Of the 242 patients, 82 (34%) had been prescribed chelation therapy during their lifetime. The median age at the time chelation therapy was first initiated was 10.4 years (range: 0.7-47.9 years). Of those who had never been transfused, 10% had received chelation therapy. Of those patients ages 1-<10 years at enrollment, 19% had been on chelation therapy starting at a median age of 2.4 years (range: 2-5 years).

Disease treatment of Amish patients differed significantly from the other patients, as these patients are typically uninsured and the cost of standard supportive care can be prohibitive. All but two Amish patients were splenectomized; none of the splenectomized patients received regular transfusions. Instead of chelation therapy, Amish patients were managed with an iron restricted diet and a combination of proton pump inhibitors and calcium citrate to reduce dietary iron uptake.

In this PK deficiency Natural History Study, the prevalence of iron overload defined by LIC was 82% in non-regularly transfused patients. Although ferritin levels correlated with LIC, ferritin levels of >1000 ng/ml had a sensitivity for LIC >3 mg/g DW of only 53%. A ferritin >1000 ng/ml is a conservative threshold for reporting the prevalence of iron overload given the specificity of 100% but likely underestimates its true prevalence in PK deficiency. Given that the ferritin >500 ng/ml had sensitivity of 90%, this is a better cut-off if ferritin is used as a screening test for selecting patients for an MRI.

Despite the conservative ferritin threshold of >1000 mg/dl, 38% of all patients who were not regularly transfused and 18% of those who were never transfused met this definition of iron overload. Furthermore, 82% of the patients who were not regularly transfused and had an MRI or chelation data had a LIC >3 g/mg DW. These data clearly show that iron overload is not limited to regularly transfused patients but is also common in patients who are not regularly transfused and even in those who have never been transfused. This is consistent with the findings in thalassemia intermedia in which iron loading occurs both in transfused and non-transfused patients.⁵

There is no consensus definition of when patients with PK deficiency are regarded as regularly transfused. Factors influencing transfusion frequency are often patient or physician dependent. Transfusion triggers vary from hospital to hospital and the degree of transfusion dependence of the patient might also differ based on characteristics, such as age, growth, and daily activities. By choosing a conservatively high cut off of at least 6 transfusions per year to distinguish between regularly and not-regularly transfused patients, we minimized the chance of confounding transfusion-related iron loading with transfusion independent iron overload.⁵⁻⁷

In this study, in non-regularly transfused patients, iron overload had also already occurred at a very young age. In non-regularly transfused patients, an MRI should be considered at the earliest age when the procedure can be done without sedation, particularly in patients with ferritin levels >500 ng/mL. In regularly transfused patients, MRI should be considered annually after one year of transfusions.

In this cohort, only five patients (7%) had cardiac iron overload by MRI. One patient in the cohort developed cardiac iron overload after only 10 lifetime transfusions. Moreover, the youngest patient with cardiac iron overload in this cohort was three years old. This indicates that the number of prior transfusions might not predict which patients are most in need of screening for cardiac iron overload.

In the non-Amish cohort, splenectomy was associated with iron loading. However, this association is not clearly causal. Since splenectomy typically occurs in the more severely affected patients with PK deficiency, other factors associated with splenectomy, such as a lower hemoglobin or increased transfusion burden, may be contributing to this relationship. Further study is needed to understand whether splenectomy is independently associated with iron loading in PK deficiency.

Although the study is biased by its retrospective nature and challenges related to rare disease registries, including variability between diagnostic and treatment standards, the results clearly show that in PK deficiency, there is a high prevalence of iron overload both in regularly transfused and not regularly transfused patients. Ferritin levels below 1000 ng/ml, hemoglobin levels above 9 g/dL, young age, and/or an absence of regular transfusions do not exclude the possibility of iron overload in patients with PK deficiency. Therefore, iron screening is important in all patients with PK deficiency and should be monitored starting in childhood. Regular monitoring for iron overload and treatment, when needed, is imperative to the management of this patient population.

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Table 1. Characteristics of non-regularly transfused patients with PK Deficiency and iron overload

	Ferritin >1000 ng/ml or chelation*			LIC >3 mg/g dry weight liver or chelation*		
	Absent (85/138, 62%)	Present (53/138, 38%)	p**	Absent (15/82, 18%)	Present (67/82, 82%)	p value**
CHARACTERISTICS						
Female sex	49/85 (58%)	24/53 (45%)	0.2	12/15 (80%)	36/67 (54%)	0.08
Amish	27/85 (32%)	13/53 (25%)	0.4	14/15 (93%)	33/67 (49%)	0.001
Age at enrollment (y)	22.6 (1.6-69.9)	38.9 (2.2-60.4)	0.005	23.4 (7.4-53.6)	34.7 (3.1- 60.4)	0.3
Hemoglobin (g/dl)	9.3 (6.2-14.1)	8.7 (6.5-12.0) n=52	0.003	9.9 (7.6-10.9)	9.0 (6.2-12.0) n=66	0.2
Absolute reticulocyte count (10 ⁶ / μ L)	0.2 (0.1-5.3) n=41	0.5 (0.1-1.2) n=15	0.2	0.9 (0.9-1.0) n=2	0.6 (0.1-0.9) n=13	0.1
Total Bilirubin (mg/dl)	3.6 (0.9-9.0) n=79	4.3 (1.3-17.6) n=48	0.01	3.5 (1.0-7.2)	3.6 (1.1-17.6) n=62	0.3
Ferritin (ng/ml)	388.0 (31.0-971.5)	1335.0 (171.5-5630.0) n=47	ND	362.5 (126.0-1065.0) n=12	969.0 (171.5-5630.0) n=53	<0.001
Liver Iron Concentration (LIC, mg/g DW)	4.0 (1.0-8.4) n=26	8.0 (2.2-33.4) n=21	<0.001	2.0 (1.0-3.0)	6.4 (2.2-33.4) n=43	ND
Transferrin saturation (%)	43.3 (8.8-100.0) n=51	62.0 (18.4-100.0) n=29	0.02	42.4 (23.2-96.6)	50.1 (12.1-100.0) n=43	0.3
Splenectomized	51/85 (60%)	47/53 (89%)	0.0002	14/15 (93%)	63/67 (94%)	1
Chelation in prior 12 months	0/85 (0%)	31/53 (58%)	ND	0/15 (0%)	31/67 (46%)	ND
Never Transfused	18/85 (21%)	4/51 (8%)	0.054	1/15 (7%)	6/65 (9%)	1
<10 lifetime transfusions	22/61 (36%)	7/39 (18%)	0.07	2/9 (22%)	9/51 (18%)	0.7
\geq 10 lifetime transfusions	39/61 (64%)	32/39 (82%)	0.07	7/9 (78%)	42/51 (82%)	0.7

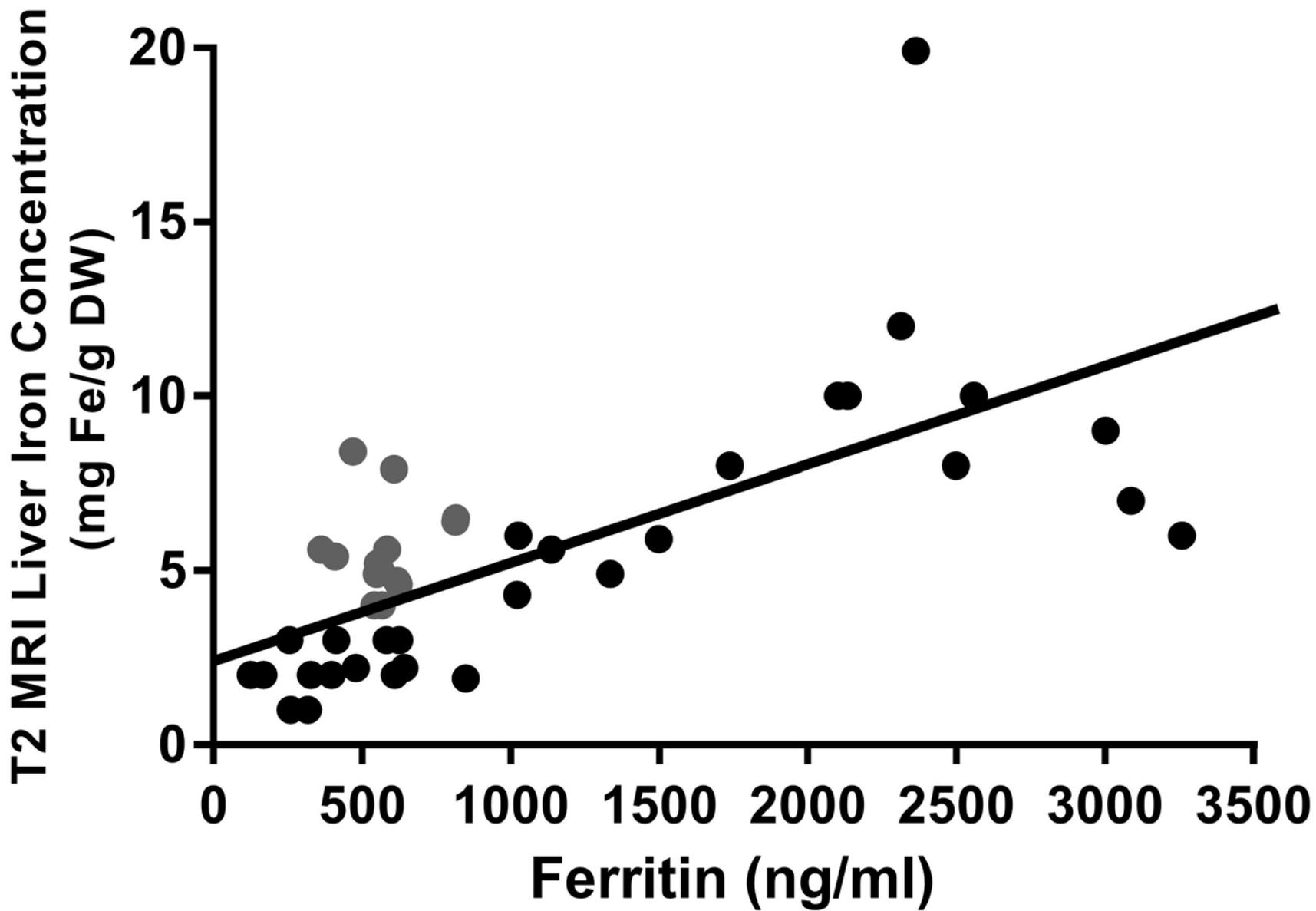
Numbers are medians (range) or absolute numbers/group total (corresponding percentage). n is shown only for those groups that do not contain the total. *Iron overload by ferritin was defined as the number of patients with ferritin >1000 ng/ml or who were treated with chelation therapy in the 12 months prior to enrollment. If a patient had >1 ferritin measurement in the 12 months prior to enrollment, the maximum ferritin value was used. Iron overload based on liver iron concentration (LIC) was defined as an LIC >3 mg Fe/g dry weight liver (DW) on T2* MRI in the 12 months prior to enrollment or who were treated with chelation therapy in the 12 months prior to enrollment. **p values of Fisher's Exact test or Wilcoxon Rank Sum test. ND: Not Done, testing is not appropriate or necessary within the same factor.

Figure Legends

Figure 1

Header: Correlation between ferritin and liver iron concentration (LIC) as measured by MRI

Legend: Correlation between ferritin and LIC ($r=0.45$, $p<0.0001$, $n=45$). Gray circles indicate the individuals with a median ferritin <1000 ng/mL but a LIC >3 mg/g dry weight liver.



SUPPLEMENTAL TABLE

Supplemental Table 1: Patient characteristics, comparing those with iron monitoring to those without monitoring, in the 12 months prior to enrollment.

	Ferritin monitoring (n=242)			MRI monitoring for liver iron assessment, in the non-Amish patient cohort (n=189)		
	Obtained in the 12 months prior to enrollment (n=175)*	Not obtained in the 12 months prior to enrollment (n=67)*	p-value**	Obtained in the last 12 months prior to enrollment (n=18)*	Not obtained in the last 12 months prior to enrollment (n=171)*	p-value**
Median*** Age at enrollment	21.8 (1.3-69.9)	16.3 (1.4-60.4)	0.3	21.1 (2.2-46.0)	19.1 (1.3-69.9)	0.5
Transfused in the 12 months prior to enrollment (%)	72/175 (41%)	9/67 (13%)	<0.0001	11/18 (61%)	67/171 (39%)	0.08
Regularly Transfused (%)	43/175 (25%)	1/67 (1%)	<0.0001	7/18 (39%)	36/171 (21%)	0.1
Median*** Hemoglobin value (g/dl)	8.8 (5.2-14.1) n=174	9.6 (6.5-13.0) n=65	0.01	8.6 (6.2-11.1)	8.7 (5.2-14.1) n=168	0.3
Splenectomized (%)	112/175 (64%)	37/67 (55%)	0.2	15/18 (83%)	83/171 (49%)	0.006

*Number of patients with known data (n) are presented in the column headers unless otherwise indicated in the table. **Using Fisher's exact test for categorical variables and Wilcoxon rank-sum test for continuous variables. ***Median presented with ranges.