Worldwide study of hematopoietic allogeneic stem cell transplantation in pyruvate kinase deficiency

Pyruvate kinase deficiency (PKD) is the most frequent glycolytic enzyme defect causing hereditary non-spherocytic hemolytic anemia.[1] PKD leads to energy deprivation of the red cell, ultimately resulting in premature red cell death. Premature red cell death causes clinical symptoms of hemolytic anemia. The degree of hemolysis can vary widely, from very mild and fully compensated forms to life-threatening anemia with transfusion dependency.[2] The treatment for PKD is mainly supportive, and consists of regular red blood cell transfusions, splenectomy and chelation therapy for iron overload.[3] Hematopoietic allogeneic stem cell transplantation (HSCT) has the potential to cure PKD. However, there is little experience of applying HSCT in PKD. The current knowledge of HSCT in PKD is predominantly based on animal studies, and guidelines are not available.[4,5] To date, only four human cases of HSCT have been published in the literature.[6-8] The total number of cases transplanted worldwide is unknown.

The aim of this study is to make a worldwide inventory of PKD cases that have been treated by HSCT, and to evaluate indication, procedures employed and outcome as a first step towards the establishment of guidelines for HSCT in PKD. In order to achieve this goal queries were sent to national and international databanks, including the European Society for Blood and Marrow Transplantation (EBMT), the Center for International Blood and Marrow Transplant Research (CIBMTR), and the National Institute of Health (NIH), as well as to physicians known to be involved in HSCT in PKD patients. For each case found, a specifically designed questionnaire was sent to the physician involved. The questionnaire contained questions on disease characteristics, pre-transplant condition, transplant regimen and post-transplant outcome.[9] All data were evaluated by an experienced physician, and institutions were contacted in case of inconsistencies. An adapted EBMT score (*i.e.*, age, donor type and donor-recipient sex combination) was calculated based on the answers provided.[10] In addition, data from two additional cases, published recently, were extracted from the literature and included.[8] To the best of our knowledge, we have included all cases worldwide.

In total, 16 cases were found to be treated by HSCT between 1996 and 2015. Patient characteristics are summarized in Table 1. Patients had all been treated in either European or Asian centers. No cases resulted as being transplanted in the USA. Patient's median age at transplantation was 6.5 years. All patients were transfusion-dependent before transplantation, with median transfusion needs of 13 units of packed red blood cells per year (range: 6 to 34 units).

Conditioning and prophylaxis characteristics are summarized in Table 1. All patients received graft-*versus*-host disease (GvHD) prophylaxis. *Ex vivo* T-cell depletion was performed in one transplant. In another, red cell depletion was performed. Five transplants were sex-matched, four were female receiver-male donor and four were male receiver-female donor; this information was not available for three cases.

Median follow-up time after transplantation was 2.3 years (range: 2 months to 19 years). Fifteen patients showed engraftment. The sixteenth patient initially showed pancytopenia and mixed chimerism. Following splenectomy six months post-transplantation, this patient's cell count spontaneously transitioned to normal with full donor chimerism. Two patients suffered from secondary graft loss; in one there was recovery to 91% donor chimerism after donor lymphocyte infusion. The outcome in the second patient was unknown.

Infectious complications and occurrence of GvHD are summarized in Table 1. The most significant infectious complications were aspergillus pneumonia (two patients), suspected aspergillus pneumonia (one patient), suspected fungal pneumonia (one patient), pneumonia (one patient), sepsis (one patient) and bacterial infection *e causa ignota* (one patient). GvHD grade 4 was reported in 6/16 cases (38%). Seven out of 16 cases (44%) did not show symptoms of GvHD. There was no correlation between GvHD prophylaxis or any other clinical factors and the occurrence of GvHD grade 2-4 in these patients.

Five out of 16 patients (31%) did not survive. All died of transplant-related causes. They had a median survival time of 13 months (range: 2-25 months). The two-year cumulative survival was 74%. Two patients had not yet reached the two-year milestone at the time of the questionnaire. The three-year cumulative survival rate was 65% (Figure 1); seven patients had not yet reached the three-year milestone.

Patients who did not survive differed significantly from surviving patients. (Figure 1, Table 2). They were significantly older (P=0.036). Nine out of ten patients (90%) <10 years of age survived transplantation, whereas two out of six (33%) ≥10 survived. Patients <10 years were less often splenectomized (P=0.001) and had lower pre-transfusion hemoglobin levels prior to HSCT (P=0.04). Patients who did not survive had all been treated in European centers. All patients treated in Asian centers survived transplantation (8/8). Patients treated in Asian centers were younger (P=0.001), less often splenectomized (P=0.041), and had lower ferritin levels prior to HSCT (P=0.048). In addition, they were more often transplanted using peripheral blood stem cells as a source (P=0.014) and more often conditioned on a cyclophosphamide regimen (P=0.007). Furthermore, patients who did not survive had frequently suffered from GvHD grade 2-4 (P=0.031). Notably, four out of five deceased patients had suffered from both GvHD grade 3-4 and infection or viral reactivation.

There were no significant differences in sex, plasma ferritin level, use of pre-transplant chelation therapy, transfusion burden in the 12 months prior to HSCT, adapted EBMT-score, conditioning regimen, relation to donor, graft type, donor-recipient sex combination, or transplant source.

In conclusion, herein we discuss the first global study on the outcome of all patients known to have undergone HSCT in PKD. Since guidelines for HSCT in PKD are lacking, this report may be a helpful first step toward future protocols. Compared to published survival rates for other forms of hereditary anemias, cohorts that are otherwise comparable in age, time period and transplant hospital, the overall survival rate after HSCT in PKD is relatively low.[11-13] The present analysis of all 16 PKD patients known to be transplanted to date showed a three-year overall survival of 65%. Significantly better survival was observed for patients transplanted before the age of ten. A negative effect of age on survival is also reported for other forms of hereditary anemia.[11,12] Concurrently, we noticed a striking difference in survival between patients treated in Asian and European centers, which could possibly be explained by the difference in age at which patients were transplanted. In addition,

Table 1. Patient characteristics.

Sex	Center	PKLR genotype	Splenectomy	N. Transf. 12mo	aEBMT	Age at HSCT	Max Ferritin	Pre. Trans. Ferritin	Trans. year	Donor	Regimen	Matching	Stem cell source	Conditioning regimen	GvHD	Infection	Outcome	Follow-up time (Mo)
Pt 1: Severe chronic anemia and progressive splenomegaly																		
M	Asia	Unknown	No	14	Good (0)	5y	-	**950**	1996	MSD	Myeloablation	**8/8**	Bone marrow	Cycph 200 mg/kg Bu 16 mg/kg *p.o.*	No	Febrile neutropenia unknown origin	Alive[%]	235
Pt 2: Concerns regarding progressive liver and heart hemosiderosis																		
F	EU	*c.[721G>T;1594C>T] p.[(Glu241*); (Arg532Trp)]*	Yes	8	Intermediate (2)	15y	-	596	2002	MFD	Myeloablation	**10/10**	Bone marrow	ATG 20 mg/kg Cycph 90 mg/kg Flu 100 mg/m² Bu 16 mg/kg *p.o.*	Grade 4 (S/G/L)	Primary CMV infection, asperg. pneum	Deceased	15
Pt 3: Transfusion dependency																		
F	Asia	*c.[1044G>T;1076 G>A] p.[(Lys348Asn);(Arg359His])*	No	14	Intermediate (2)	1y, 7mo	3357	206	2009	Cord	Myeloablation	**7/8**	Cord blood	ATG 7.5 mg/kg Cycph 200 mg/kg Bu 19.2 mg/kg	Grade 1 (S)	Bacterial infection	Alive	72
Pt 4: High transfusion dependency and secondary hemochromatosis																		
F	EU	*c.[721G>T;1463G>A] p.[(Glu241*);(Arg488Gln)]*	No	10	Intermediate (2)	3y	**2444**	**1161**	2009	MUD	Non-myeloablation/ RIST		Bone marrow	ATG 30 mg/kg Flu 160 mg/m² Thio 8 mg/kg Treo 42 mg/kg	No	No	Alive	65
Pt 5: Transfusion dependency																		
M	Asia	*c.[119G>A;1015G>A] p.[(Arg40Gln);Asp339Asn)]*	No	13	Intermediate (1)	2y, 6mo	-	-	2009	MUD	Non-myeloablation/ RIST	**8/10**	Peripheral blood	ATG 15 mg/kg Cycph 200 mg/kg Flu 120-160 mg/m² Bu 3.2-4.8 mg/kg	Grade 2 (S)	Fever unknown origin 8	Alive	8
Pt 6: Transfusion dependency																		
F	EU	*c.[1123_1133dup11; 1123_1133dup11] p.[(Met377fs;Met377fs)]*	Yes	20	Intermediate (1)	17y	1888	1888	2010	MFD	Myeloablation	**8/8**	Peripheral blood	Cycph 120 mg/kg Bu 12.8 mg/kg	Grade 4 (S, G)	E. faecium sepsis, susp. fungal pneumonia	Deceased	5
Pt 7: Progressive transfusion dependency, decreasing quality of life																		
F	EU	*c.[494G>T;1529G>A] p.[(Gly165Val);(Arg510Gln)]*	Yes	12	Intermediate (2)	39y	**1311**	**650**	2011	MUD	Non-myeloablation/marrow RIST	**8/8**	Bone	ATG 600 mg/m² Flu 120 mg/m² Bu 10.8 mg/m²	Grade 4 (S,G,L)	No	Deceased	25
Pt 8: Transfusion dependency																		
F	EU	*c.[1532G>A;1612G>T] p.[(Gly511Glu);(Glu538*)]*	Yes	8	Intermediate (2)	7y	**771**	**771**	2013	MFD	Non-myeloablation/ RIST	**10/10**	Bone marrow	ATG 4 mg/kg Flu 160 mg/m² Thio 8 mg/kg Treo 42 mg/m²	No	CMV reactivation	Alive	29

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Pt 9: Transfusion dependency																		
M	EU	*c.[1481T>C;1675C>T] p.[(Ile494Thr);(Arg559*)]*	No	12	Intermediate (1)	6 y	**675**	**675**	2013	MUD	Non-myeloablation/ RIST	**9/10**	Bone marrow	ATG 6 mg/kg Flu 160 mg/m² Thio 8 mg/kg Treo 42 mg/m²	Grade 4 (S, G,L)	CMV and EBV reactivation	Deceased	2
Pt 10: Transfusion dependency																		
M	Asia	*c.[848T>C;941T>C] p.[(Val283Ala);(Ile314Thr)]*	No	13	Intermediate (1)	1y, 6mo	-	593.5	2013	MUD	Myeloablation	**9/10**	Peripheral blood	ATG 15 mg/kg Cycph 200 mg/kg Flu 120-160 mg/m² Bu 3.2-4.8 mg/kg	Grade 4 (G)	No	Alive	34
Pt 11: Transfusion dependency, problems with iron overload treatment due to compromised renal function																		
M	EU	*c.[1618+37_2064del; 1618+37_2064del] p.[(Lys541fs);(Lys541fs)]*	Yes	6	Intermediate (1)	10y	4149	**7026**	2014	MUD	Myeloablation	**9/10**	Bone marrow	ATG 8 mg/kg Flu 160 mg/m² Bu Targeted dose, target AUC 90	Grade 3 (S,G)	Asperg. pneum.	Deceased	13
Pt 12: Transfusion dependency																		
M	Asia	*c.[661G>T;941T>C] p.[(Asp221Tyr);(Ile314Thr)]*	No	9	Intermediate (1)	9mo	-	-	2014	Cord	Myeloablation	**7/10**	Cord blood	ATG 15 mg/kg Cycph 200 mg/kg Flu 120-160 mg/m² Bu 3.2-4.8 mg/kg	Grade 4 (unknown)	Pneumonia	Alive	24
Pt 13: Transfusion dependency																		
M	Asia	*c.[848T>C;848T>C] p.[(Val283Ala);(Val283Ala)]*	No	13	Intermediate (1)	1y, 2mo	-	297.3	2015	MUD	Non-myeloablation/ RIST	**10/10**	Peripheral blood	ATG 15 mg/kg Cycph 200 mg/kg Flu 120-160 mg/m² Bu 3.2-4.8 mg/kg	No	No	Alive	12
Pt 14: Transfusion dependency, secondary hemochromatosis and hepatocarcinoma																		
M	EU	*c.[993C>A;1015G>C] p.[(Asp331Glu);(Asp339His)]*	Yes	34	Intermediate (2)	41y	-	**1650**	2015	MSD	Myeloablation		Bone marrow	ATG 30 mg/kg Flu 160 mg/m² Thio 8 mg/kg Treo 42 mg/kg	No	Susp. asperg. pneum.	Alive	12
Pt 15: Transfusion dependency, secondary hemochromatosis, spinal compression fracture due to osteoporosis																		
M#	Asia	*c.[1270-3C>A;1618G>T] p.[(?);(Gly540*)]*	Yes	-	-	11y	-	2000	-	MUD	Myeloablation	**9/10**	Peripheral blood	ATG 7.5 mg/kg Cycph 200 mg/kg Flu 120 mg/m²	No	-	Alive	36
Pt 16: Transfusion dependency																		
F#	Asia	*c.[1270-3C>A;1618G>T] p.[(?);(Gly540*)]*	No	-	-	8y	-	-	-	MUD	Myeloablation		Peripheral blood	ATG 7.5 mg/kg Cycph 200mg/kg Flu 200 mg/m²	No	-	Alive	30

#Data retrieved from Kim, 2016[8]

%At last follow up

M: male; F: female; Y: years; Mo: months, N. Transf. 12mo: estimated number of red blood cell transfusions in 12 months prior to HSCT; Max. Ferritin: maximum ferritin reported in ng/mL; Pre. Trans. Ferritin; pre-transplant ferritin level in ng/ml, (ferritin levels in bold: under chelation regimen); MUD: matched unrelated donor; MSD: matched sibling donor; MFD: matched family donor; Cord; cord blood; ATG: anti-thymocyte globulin; Flu: fludarabine; Bu: busulfan; Thio; thiothepa; Treo: treosulfan; Cycph: cyclophosphamide; - : unknown; RIST: reduced-intensity hematopoietic stem cell transplantation; S: skin; G: GI tract; L: liver; CMV: cytomegalovirus: EBV: Epsteinn-Barr virus; susp: suspected; asperg: aspergillus; pneum: pneumonia; E. faecium sepsis: enterococcus faecium sepsis; GvHD: graft-*versus*-host disease; HSCT: hematopoietic allogeneic stem cell transplantation; aEBMT :adapted European Society for Blood and Marrow Transplantation score; *p.o.: per os*.

Asian patients were non-splenectomized in many instances, and had lower pre-transplantation ferritin levels, which could also be related to the young age at which HSCT was performed.

Asian patients were more frequently transplanted with peripheral blood stem cells as opposed to bone marrow-derived stem cells. Peripheral blood stem cells are easier to collect from the donor, but reportedly increase the risk of chronic GvHD.[14] Our cohort, however, was too small to analyze the specific effect of stem cell source on the occurrence of chronic GvHD.

An important limitation of this study is its retrospective character, and the fact that the small sample size did not allow us to perform *post hoc* correction for multiple testing. Therefore, the quantitative analysis of this data should be interpreted with care. Other limitations include the heterogeneity of conditioning regimens, and heterogeneity in the pre-transplant risk classification systems used. However, we did observe a better survival for patients transplanted prior to age ten. This effect of age might also play a role in the observed differences in survival between patients treated in European centers and those treated in Asian centers.

Although HSCT should be considered an investigational treatment, the strong decline in survival of treated patients over the age of ten suggests the need to evalu-

Table 2. Statistical differences between surviving and non-surviving patients.

	Survivor	Non-survivor	*P* value
Age in years	7.5 – 3.0 (0.8-41)	17.4 – 15.2 (6-39)	0.036*
Asian hospital	8/11 (73%)	0/5	0.026*
Splenectomy performed	3/11 (27%)	4/5 (80%)	0.106
Mean Hb (g/dL) (N=13)	6.0 – 5.5 (4,5-7,9)	7.1 – 6.9 (6.0-8.1)	0.112
Pre-transplant ferritin (ng/ml) (n=12)	804 – 771 (206-1650)	2167 – 675 (596-7026)	0.432
Myeloablation	6/11 (55%)	4/5 (80%)	0.588
Graft type			0.507
MSD	2/11 (18%)	0/5	
MUD	6/11 (55%)	3/5 (60%)	
CORD	2/11 (18%)	0/5	
MFD	1/11 (9%)	2/5 (40%)	
Transplant source			0.333
Bone marrow	4/11 (36%)	4/5 (80%)	
Peripheral blood	5/11 (45%)	1/5 (20%)	
Cord blood	2/11 (18%)	0/5	
GvHD			0.015*
None	7/11 (64%)	0/5	
Grade 1	1/11 (9%)	0/5	
Grade 2	1/11 (9%)	0/5	
Grade 3	0/11	1/5 (20%)	
Grade 4	2/11 (18%)	4/5(80%)	

(descriptive statistics: mean – median (range) (N), frequencies number/total (percentage)
***P*<0.05**

Continuous variables were expressed as mean, median and range, and subgroups were compared using Mann-Whitney U tests. Categorical data was compared using Fisher's exact test for binomial and the Fisher-Freeman-Halton exact test for contingency tables larger than 2x2. Statistical significance was considered as $P \leq 0,05$. All tests were two-sided. Post hoc multiple comparison correction was not applied. Graft-*versus*-host disease (GvHD) is defined and graded according to international criteria.[15] Pre-transplant laboratory results from splenectomized patients are from the period after splenectomy. Hb: hemoglobin; MUD: matched unrelated donor; MSD: matched sibling donor; MFD: matched family donor; Cord; cord blood.

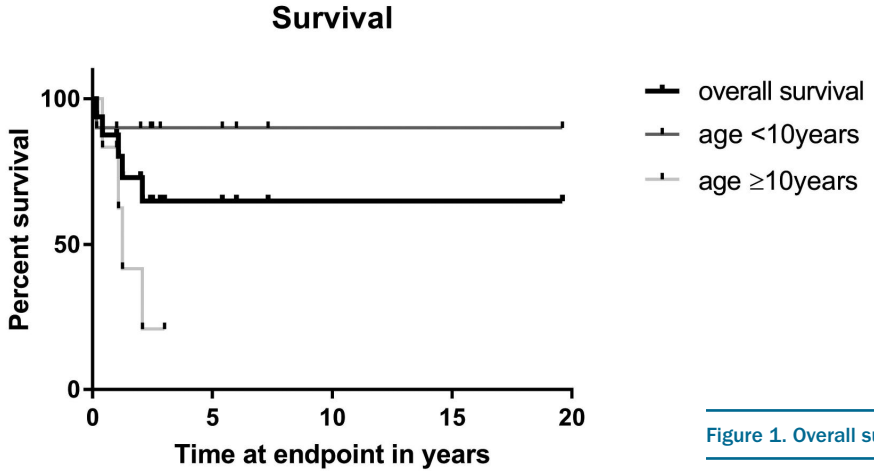

Figure 1. Overall survival, according to age.

ate HSCT as a treatment option early in life. However, since the rate of grade 3-4 GvHD was relatively high (7/16 = 44%), and death resulting from GvHD was likewise high (5/16 = 31%), transfusion dependency alone should not be an indication for performing HSCT in PKD.

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