Hematopoietic stem cell transplantation for patients with paroxysmal nocturnal hemoglobinuria previously treated with eculizumab: a retrospective study of 21 patients from SFGM-TC centers.

Paroxysmal nocturnal hemoglobinuria (PNH) is a rare acquired hematopoietic stem cell disorder which manifests with hemolysis (classical PNH) due to loss of expression of the CD55 and CD59 proteins, which leads to complement mediated cell lysis.[1] Despite being the only curative treatment for PNH,[2] allogeneic hematopoietic stem cell transplantation (HSCT) is not recommended as first-line therapy for classical PNH (C-PNH) or for patient with past thrombosis considering the safety and efficacy of Eculizumab.[3,4] The European society for Blood and Marrow Transplantation (EBMT) previously reported 30% overall mortality in PNH patients transplanted between 1978 and 2007, with an unacceptable higher risk of mortality in patients with pre-transplant thrombosis history.[5] Nowadays, HSCT might thus be considered, in absence of alternative treatment, for 5 to 10% of patients with C-PNH who evolve to myelodysplastic syndromes (MDS) or acute myeloid leukemia[4,6] and for 2 to 4% patients with recurrent thrombosis under eculizumab.[4,7] HSCT might also be questioned in 1%

Table 1. Characteristics of patients and transplantations.

Indication for HSCT	#	Sex	Time between diagnosis and HSCT (years)	Age at HSCT (years)	Conditioning regimen*	Donor	SC	GvHD prophylaxis	Acute GvHD Localisation (stage)	Relapse	Death	Follow up (months)
Clonal evolution to MDS	1[†]	**M**	**0.8**	**67**	**NMA+ATG**	**MRD**	**PB**	**CsA**	**Skin (1)**	**Yes**	**Yes[d]**	**72**
Recurrent thrombosis	2	**M**	**1**	**19**	**MAC+ATG**	**MRD**	**BM**	**CsA, MTX**	**No**	**No**	**No**	**96**
Aplastic anemia	3	F	0.3	23	NMA+ATG	MRD	BM	CsA, MTX	No	No	No	107
	4	M	5	46	NMA+ATG	MRD	PB	CsA, MTX	No	No	No	45
PNH	5	F	11	30	NMA+ATG	MRD	PB	CsA, MTX	No	No	No	39
	6	F	0.8	18	NMA+ATG	MRD	BM	CsA, MMF	Skin (4)	No	Yes[e]	4
	7	F	6	27	NMA+ATG	MUD	PB	CsA, MTX	GI (3)	No	No	91
	8[†]	M	6	34	NMA+ATG[a]	MUD	BM	Tac, MTX	No	No	No	79
	9[†]	M	3	53	NMA+ATG	MUD	BM	MTX	No	No	Yes[e]	1
	10	F	7	36	NMA	MUD	PB	CsA, MTX	No	No	No	112
	11[†]	F	9	30	NMA+ATG[a]	MMUD	CB	CsA	No	No	Yes[e]	5
	12[‡]	**F**	**4**	**29**	**NMA[a]**	**MMUD**	**CB**	**CsA, CS**	**No**	**No**	**No**	**120**
	13[†]	M	19	34	NMA[a]	Haplo	BM	CsA, MMF	No	No	No	2
Transfused classical PNH	14	M	4	38	NMA+ATG	MRD	BM	CsA, MMF	Skin (1)	No	No	43
	15	F	9	28	NMA+ATG	MRD	BM	CsA, MTX	No	Yes	No	30
	16	**F**	**4**	**50**	**NMA**	**MRD**	**PB**	**CsA, MMF**	**GI (4), Skin (2)**	**No**	**Yes[f]**	**12**
	17[b]	**M**	**0.6**	**19**	**NMA+ATG** **MAC+ATG** **NMA[a]**	**MRD** **MRD** **MMUD**	**BM** **PB** **CB**	**CsA, MMF** **CsA, MMF** **CsA, MMF**	**No**	**No**	**No**	**107**
	18[†]	F	1	27	NMA+ATG[a]	MUD	BM	CsA, MTX	Skin (1)	No	No	103
	19[†c]	F	16	26	NMA+ATG[a]	MUD	BM	CsA	GI (4), Liver (2)	No	Yes[f]	3
					No	MUD	PB	CsA, MMF				
Non-transfused classical PNH	20	F	3	58	NMA	Syng	BM	No	No	No	No	53
	21	F	2	19	MAC	Syng	PB	No	No	No	No	72

Chronic graft-*versus*-host disease (GvHD) is not represented as it was not observed in this cohort; bold lines represent 5 (23.8%) patients with thrombosis treated before hematopoietic stem cell transplantation (HSCT): localisations were hepatic veins in patient #16 and #17, mesenteric veins in #1, #12 and #2, pulmonary veins in #2 and renal artery in patient #1; *For detailed conditioning regimen see *Online Supplementary Table S1*; [†]Patients who received 1 immunosuppressive treatment (association antithymocyte globulin plus cyclospoirine) before HSCT; [‡]Patients who received 2 immunosuppressive treatments (association antithymocyte globulin plus cyclospoirine) before HSCT; [a]Conditioning contained 2 Gy total body irradiation; [b]Patient #17 received 3 HSCT for primary failure to engraftment ; [c]Patient #19 received a second HSCT for secondary graft failure; [d]Cause of death was relapsing disease; [e]Cause of death was fungal infection; [f]Cause of death was acute GvHD; MAC: myeloablative conditioning; NMA: non myeloablative conditioning; ATG: antithymocyte globulin; SC: stem cell source; Syng: syngeneic donor; MRD: HLA-matched related donor; MUD: HLA-matched unrelated donor; MMUD: HLA-mismatched unrelated donor; Haplo: haploidentical donor; BM: bone marrow; PB: peripheral blood; CB: cord blood; CsA: Cyclosporine A; MMF: mycophenolate mofetil; MTX: methotrexate; Tac: tacrolimus; CS: corticosteroid; GI: gastro-intestinal.

who may evolve to AA-PNH4 or for 34 to 51% of C-PNH patients who are still transfused under eculizumab.[7–11] To date, data on the outcome of HSCT for patients who were previously treated with Eculizumab as well as best management of anti-C5 therapy in the context of HSCT are scarce.[12] We report herein the outcome of 21 patients, previously treated with Eculizumab, who underwent HSCT between 2007 and 2017. We show that regardless of the indications for HSCT in PNH patients previously treated with Eculizumab, HSCT is still associated with almost 30% of mortality mainly due to infections and acute graft-*versus*-host disease (GvHD).

Patients were identified and data were collected through the French PNH registry[4] and the registry from the *Société Francophone de Greffe de Moelle et de Thérapie Cellulaire*. An additional questionnaire, which focused on the management of Eculizumab at time of transplantation, was sent to investigators (*Online Supplementary Data*). Infectious and GvHD complications were graded according to the commonly used scales.[13,14] Anti-fungal prophylaxis was conducted according to local policy. Data were described through proportions or median with inter-quartile range (IQR: 25%-75%). Statistical analyses were conducted using SPSS. Fine and Gray's model of competing-risks regression adjusted according to death, relapse and non-engraftment incidence of acute GvHD (aGvHD) was performed using R Software.[15]

Patients' and transplantations' characteristics are detailed in Table 1 and *Online Supplementary Table S1*. Indication for HSCT were clonal evolution to a MDS in 1 (4.7%) patient, recurrent thrombosis under Eculizumab in 1 (4.7%) patient, AA-PNH in 11 (52.4%), transfused C-PNH in 6 (28.6%), and non-transfused C-PNH with a syngeneic donor available in 2 (9.5%) patients. Before HSCT, Eculizumab was infused at the dose of 600 mg every week for 4 weeks followed by infusions of 900 mg every 14 days for every patient. Median duration of Eculizumab was 8 months (IQR: 5-21) and no dose modifications was reported. Among transfused C-PNH, median duration of Eculizumab was only of 6.5 months (IQR: 5-17.75). ABO group incompatibility (4 minor, 3 major, 2 major and minor) and sex-mismatched (female to male in 4 cases) were observed in 9 (42.9%) and 13 (61.9%) patients, respectively.

Among the 21 patients, 20 (95.2%) patients engrafted after HSCT. Median time to engraftment was 20.5 days (IQR: 13.5-23.75). Chimerism was assessed in 17 patients (2 syngeneic transplantations and #16 were not assessed while patient #9 died before evaluation). Full donor and mixed chimerism were observed in 10 (58.8%) and 6 (35.3%) patients, respectively. Patient #17 failed to engraft two times and eventually switched to full donor chimerism at day+27 after a third HSCT. Despite engraftment at day+12, patient #19 received a second transplant for graft failure. Overall, Eculizumab did not seem to interfere with engraftment when compared to 93% engraftment rate of the EBMT cohort.[5]

During follow up, 7 patients developed aGvHD. Among the 19 patients at risk (2 syngeneic transplantations were excluded), cumulative incidence function (CIF) of aGvHD was 38.1% [±standard error (se) 11.9%]. The median time to onset was 50 days (IQR: 19.50-91.75). Grade III-IV was reached by 4 patients. The overall rate of aGvHD was comparable to the CIF of 40% observed within the EBMT cohort.[5] Transfused C-PNH and AA-PNH CIF of aGvHD were 66.6% (±se 24.2%) and 19.3% (±se 13.1%), respectively (P=0.04). In our cohort, transfused C-PNH were exposed to an increased risk for aGvHD. Among the 16 patients at risk, chronic GvHD (cGvHD) was not observed (patients #9 and #19 died before 100 days, patient #13 did not reach day 100 at last follow up and the 2 syngeneic transplantations were excluded for this analysis). Contrariwise, EBMT reported cGvHD CIF of 29% at 5 years. Among those 16 patients, few had risk factors of cGvHD: 6 (37.5%) presented aGvHD, 12 (75%) received ATG in the conditioning regimen, and only 2 (12.5%) patients received HSCT from a HLA-mismatched unrelated donor. It is difficult to draw robust conclusions on the role of Eculizumab in this small series.

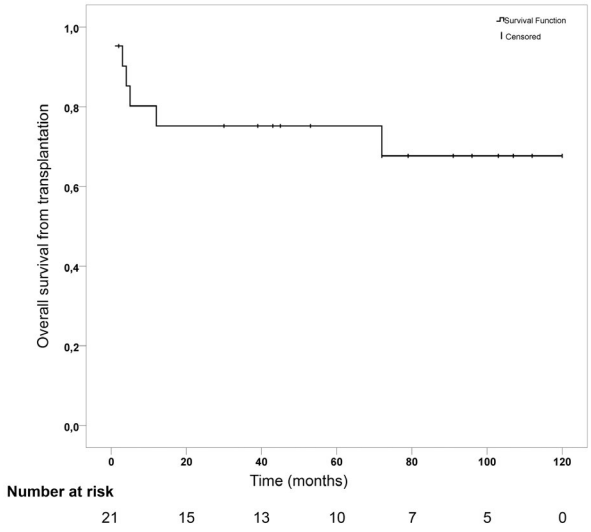

Figure 1. Kaplan-Meier survival curve for overall survival from transplantation.

Infectious complications were the main problem in this population. Fatal fungal infections were observed in 3 (14.3%) patients (#6, # 9, #11). Maximum grade for bacterial infections was 4 in 2 (9.5%) patients (#15, #3). One grade 4 viral infection was documented in one patient (#8) after day 100 (VZV meningitis). Grade 3 fungal (pulmonary aspergillosis), bacterial and viral infections were documented in 1 (4.8%), 8 (38.1%) and 3 (14.3%) patients, respectively. Anti-fungal prophylaxis was not homogeneous between centers and thus cannot be analyzed carefully in such a small number of patients. We observed a significant association between the use of ATG and grade 3 to 5 infections (P=0.01). Statistical association between ATG and infections should be interpreted with caution considering the size of this population. The high rate of infectious complications was expected as i) infectious complications were the main cause of mortality in the EBMT cohort,[5] ii) 11 (52.3%) patients received HSCT for AA-PNH, and iii) the known general susceptibility to infection in PNH.[4,11] However, there were no fatal infections in C-PNH under Eculizumab from the French PNH registry,[4] which highlights the increased risk of fatal infections for transfused C-PNH who may receive HSCT, and the need to closely monitor those patients after transplantation for fungal infections. Finally, patient #9 experienced thrombosis while PNH clone was negative.

With a median follow-up time of 53 months (IQR: 8.5-99.5), Kaplan-Meier estimated cumulative overall survival (OS) at 6 years was 67.7% (±se 11.2%) (Figure 1).

Among the 6 events, causes of death were infections, as mentioned above, in 3 (50.0%) patients, aGvHD in 2 (33.3%) patients, and relapse of MDS in 1 (16.7%) patient. OS at 2 years in AA-PNH and transfused C-PNH were 72.7% (±0.13) and 66.7% (±0.19), respectively. With a median follow-up time of 5 years, the 2 syngeneic HSCT were well tolerated and are alive at the time of this report. Relapse occurred in 2 (9.5%) patients: patient #1 relapsed at 8 months from his MDS and patient #15 relapsed at 2 months with recurrent hemolysis. Thus OS (67.7% *versus* 68%) and causes of death (50% *versus* 54.7% of infections and 33.3 *versus* 28.1% of GvHD) are comparable to the EBMT cohort that focused on non-Eculizumab treated patients transplanted before 2007.[5] The 6-years OS of non-transplanted C-PNH patient under Eculizumab was 92% in the French PNH registry.[4] Therefore, this may question the role of HSCT in transfused C-PNH under Eculizumab in absence of a syngeneic donor.

Regarding Eculizumab management before HSCT, the last drug administration was in the month preceding transplantation in 18 (85.7%) patients. Among these 18 patients, 16 received Eculizumab during the conditioning regimen. Patients #3 and #7 stopped Eculizumab 2 and 4 months, respectively, before HSCT due to evolving AA-PNH, and patient #13 at 54 months because he was lost to follow up. After HSCT, 18 (85.7%) patients discontinued Eculizumab after engraftment without experiencing signs of hemolysis. Eculizumab was given after HSCT for 3 (14.3%) patients. Patient #11, who presented hemolytic crisis at day+27, was successfully treated with one dose of Eculizumab 900mg. Patient #17 received infusions of 900 mg every two weeks for 2.6 months due to engraftment failure (Eculizumab was discontinued after the third transplantation's engraftment). For patient #15, Eculizumab was reintroduced due to disease relapse, which is still the case today. Thus, best time for last infusion of Eculizumab seems to be during the conditioning regimen. As expected, administration of the drug is mandatory in case of autologous hematopoiesis (relapse, graft failure or non-engraftment).

To conclude, this study, although retrospective, gives insight into the outcome of transplanted PNH patients in the context of complement blocker. Best timing for last drug infusion seems to be during the conditioning regimen. Allogeneic HSCT for C-PNH was associated with a high rate of aGvHD. Median duration of Eculizumab in transfused patients referred to HSCT was short. Eculizumab seems not to change the risk of HSCT complications in PNH patients, who are still associated with toxicities once referred to HSCT. Therefore, HSCT after complement blocker should only be proposed in the absence of alternative treatment and after careful assessment of the risk-benefit ratio, especially in transfused C-PNH patients.

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doi:10.3324/haematol.2017.182360

Information on authorship, contributions, and financial & other disclosures was provided by the authors and is available with the online version of this article at www.haematologica.org.

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