Allogeneic stem cell transplantation in paroxysmal nocturnal hemoglobinuria

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ABSTRACT

Background
In the era of eculizumab, identifying patients with paroxysmal nocturnal hemoglobinuria who may benefit from allogeneic stem cell transplantation is challenging.

Design and Methods
We describe the characteristics and overall survival of 211 patients transplanted for paroxysmal nocturnal hemoglobinuria in 83 EBMT centers from 1978 to 2007. Next, we conducted a comparison with a cohort of 402 non-transplanted patients with paroxysmal nocturnal hemoglobinuria diagnosed between 1950 and 2005 in 92 French centers. We compared the occurrence of complications (i.e. thromboembolism and aplastic anemia) using either an individual or a stratum-matching procedure.

Results
After a median follow-up of 5 years, the 5-year overall survival rate ± standard error (%) was 68±3 in the transplanted group (54±7 in the case of thromboembolism, 69±5 in the case of aplastic anemia without thromboembolism and 86±6 in the case of recurrent hemolytic anemia without thromboembolism or aplastic anemia). Only thromboembolism as the indication for transplantation was associated with worse outcome (P=0.03). We identified 24 pairs of transplanted and non-transplanted patients with thromboembolism for the matched comparison, with worse overall survival for the transplanted patients (hazard ratio=10.0; 95% confidence interval, 1.3-78.1; P=0.007). This was confirmed by the global matching procedure (P=0.03). As regards aplastic anemia without thromboembolism, 30 pairs were identified for the matched comparison. It was not observed that transplanted patients had a significantly worse overall survival (hazard ratio=4.0; 95% confidence interval, 0.9-18.9; P=0.06). A global matching procedure was not feasible.

Conclusions
Allogeneic stem cell transplantation is probably not a suitable treatment option for life-threatening thromboembolism in paroxysmal nocturnal hemoglobinuria.

Key words: paroxysmal nocturnal hemoglobinuria, hematopoietic stem cell transplantation.

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Introduction

Paroxysmal nocturnal hemoglobinuria (PNH) is a rare acquired hematopoietic stem cell disorder, related to a somatic mutation in the phosphatidylinositol glycan class A (*PIG-A*), X-linked gene, leading to deficient expression of glycosyl phosphatidylinositol–anchored proteins. The disease is characterized by hemolytic anemia, marrow failure, or venous thrombotic events (TE). The pleiomorphic clinical presentation of PNH has led to two recognized entities: one, predominantly hemolytic without overt marrow failure, is referred to as classical PNH[1] and the other, with marrow failure, is often described as the aplastic anemia - PNH syndrome (AA-PNH).[2] Thromboses remain the major life-threatening complication in each disease subcategory.[3,4]

Recent studies have focused on inhibiting the complement cascade, using eculizumab, a humanized anti-C5 monoclonal antibody, in patients with hemolysis.[5,6] Eculizumab significantly reduces the risk of TE by inducing a substantial and sustained decrease in the activation of both the plasma hemostatic system and the vascular endothelium,[7] likely contributing to its protective effect on the risk of thromboembolism.[8] Moreover, it has recently been suggested that eculizumab improves survival.[9] Patients with severe aplastic anemia (SAA) with or without a PNH clone are currently treated with either allogeneic stem cell transplantation (SCT) or immunosuppressive therapy depending on the patient's age and availability of a suitable human leukocyte antigen (HLA)-matched hematopoietic stem cell donor.[10]

The only curative treatment for PNH is SCT. *In vitro* and *in vivo* studies have shown that PNH cells can be eradicated following SCT.[11] However, the risk of treatment-related mortality after SCT is relatively high, with graft-*versus*-host disease (GvHD) accounting for most of the transplant-related deaths. There are a limited number of single-center studies on SCT for PNH[12-21] (reviewed by Parker[1] and Matos-Fernandez[22]) and only one registry study involving more than 50 patients.[23] The decision to perform a SCT in PNH has usually been deferred until disease progression to recurrent, life-threatening thromboembolism, refractory or transfusion-dependent hemolytic anemia, or development of SAA.[1,22]

We evaluated the outcome and risk factors affecting survival after allogeneic SCT in the largest cohort ever studied of 211 PNH patients who were reported to the European Group for Blood and Marrow Transplantation (EBMT). Moreover, we conducted a formal survival comparison of this cohort of transplanted PNH patients with a matched cohort of non-transplanted PNH patients reported to, and previously published by, the French Society of Hematology (SFH).[3,4]

Design and Methods

Data collection

This retrospective multi-center study was conducted through the SAA Working Party (SAAWP) of the EBMT and the SFH (*Online Supplementary Appendix*). The EBMT maintains a registry in which participating centers report consecutively transplanted patients. Data include demographic information, pre-transplant treatment, transplant date, type, source of transplantable cells and protocols for transplantation, transplant outcomes, status at latest follow-up and cause of death. From 1978 to 2007, data on 211 patients transplanted for PNH in Europe were reported to the EBMT by 83 centers. The diagnosis of PNH was made on the basis of an unequivocal positive Ham test[24] and/or by flow cytometry (more than 5% of cells deficient in glycosyl phosphatidylinositol–anchored proteins).[25] An additional questionnaire was sent to all centers with PNH patients requiring more specific details regarding PNH history: pre-transplant treatment, PNH clone size at the time of transplantation, indication for the transplant (AA, recurrent hemolytic crises or TE) and type of PNH at the time of the transplant (classical PNH or AA-PNH syndrome). All data were carefully checked and the institutions' physicians were contacted (by RPL and/or JYM) if there were any inconsistencies. Previously reported data on 402 non-transplanted patients, diagnosed between 1950 and 2005, recorded from 92 SFH centers,[3,4] were used for comparison. The study was carried out in accordance with the Declaration of Helsinki. EBMT registration requires informed consent by the patient; use of the SFH cohort's data was approved by the review board of the SFH.

Statistical analysis

Transplanted patients with paroxysmal nocturnal hemoglobinuria

The characteristics of the patients and transplants were described through proportions or median and inter-quartile range (IQR). Engraftment was defined as achieving an absolute neutrophil count of 0.5×10^9/L for at least 3 consecutive days. Acute GvHD and chronic GvHD were defined and graded according to previously published criteria.[26] The cumulative incidence function (CIF), with death as a competing event, was used to estimate acute and chronic GvHD.[27] With regards to the CIF of acute GvHD, when the date of the event relative to the graft was unknown (26 patients), the time to GvHD was randomly selected between 10 and 100 days using a uniform distribution. Survival was calculated from the date of transplantation to the date of last follow-up or date of death from any cause. The survival rates were calculated by the Kaplan-Meier method in the overall population and in the subgroups of patients according to SCT indication (TE, AA without TE).[28] The following variables were tested as prognostic factors: age, sex matched between donor and recipient, interval from diagnosis to transplant, year of transplantation, stem cell source, donor type (sibling *versus* unrelated), indication for SCT (AA, recurrent hemolytic crisis or TE) and classification of the disease (classical PNH or AA-PNH). All factors were assessed separately using the log-rank test and results expressed through hazard ratios (HR) with 95% confidence intervals (95% CI) derived from the proportional hazard model.[29,30] All factors with a $P<0.30$ in the univariate analysis were analyzed in the multivariate model using proportional hazards regression. All models were built using backward selection and the likelihood ratio test.[30] The same type of analysis was performed separately in patients grafted for TE and in those transplanted for AA without TE. Only factors that reached a $P\leq0.05$ were retained in the final model.

Survival comparison between transplanted and non-transplanted patients with paroxysmal nocturnal hemoglobinuria

The comparison was performed from the time of a severe (life-threatening) complication occurring among patients who experienced the same type of complication (TE or AA without TE). Among patients having experienced one of these complications, two matching procedures were used to select non-grafted patients comparable to grafted patients. First, a one to one matching procedure (*matched pair analysis*) was attempted using the following criteria, selected *a priori*: severity of the complication, age at complication and year of complication, delay between PNH diagnosis and the occurrence of complications. In addition, the survival time

from complication for a matched non-transplanted patient had to be longer than the time interval from the complication to SCT for the corresponding transplanted patient. In a second step, a stratum matching procedure (*global matching*) was undertaken using the same prognostic factors to define strata related to survival in at least one of the two groups (grafted and non-grafted patients). Patients who developed myelodysplastic syndrome before AA or TE were excluded. Non-grafted patients who died within 3 months after a complication were also excluded, since they might have been considered scheduled for SCT but could not have received it.

For the one to one matching, TE was distinguished into three classes of severity: Budd-Chiari and/or central nervous system thrombosis, other thromboses except phlebitis, and phlebitis. Age at TE was matched at ± 10 years, year of TE at ± 15 years and time interval between TE and diagnosis on category defined as less than 1 month, 1 or 3 months, 3 to 6 months, 6 months or more. Three groups were defined for aplastic anemia; patients who did not receive immunosuppressive therapy, patients transplanted from HLA-identical siblings and patients who received an unrelated transplant for AA. Age at AA and year of AA were matched as for TE. The time interval between PNH and AA was defined as more than 6 months before diagnosis, between 6 months before and 6 months after diagnosis and 6 months or more after diagnosis. One to one matching of a non-grafted patient to a grafted patient was performed in order to select the maximum number of pairs in the analysis. The difference in overall survival (OS) between grafted and non-grafted patients was tested through a proportional hazards model, stratified on each matched pair, and expressed as a hazard ratio with 95% confidence interval of grafted patients relative to non-grafted patients. In addition, OS was compared through a proportional hazards model between selected and non-selected patients among grafted and non-grafted patients, separately, to check that patients in the matched comparison did not differ from the total population.

For the stratum matching procedure, the following factors were tested: severity of complication, age at and year of complication per decade, interval between the complication and PNH diagnosis defined in categories as for the one to one matching procedure. The relation to survival was tested through a proportional hazards model separately in each group. Starting from the most predictive survival factor, distribution of this factor across the two groups was examined and categories with no or very few patients (≤4) in one of the two groups were deleted. The previous prognostic factors were then searched for and the same procedure was applied to the successive predictive factors. At the end of this procedure, a combination of levels of the factors still related to survival in one of the two groups defined a matching stratum for the survival comparison between the two groups. This comparison was first performed by using a proportional hazards model stratified on strata, assuming that the hazard ratio among grafted patients relative to non-grafted patients did not vary across strata; second, the survival analysis was performed with the same proportional hazards model using as covariates, treatment, strata, interaction between treatment and strata, allowing us to test whether the hazard ratios among grafted patients relative to non-grafted patients vary from one stratum to the other.

In all survival analyses using a proportional hazards model, continuous factors were categorized using systematic limits defined approximately in quintiles (roughly 20th, 40th, 60th and 80th percentiles). If the relative death rates (ratio of the observed death rate in each category to the expected death rate, assuming no variation of death rate across categories) in two or more adjacent categories were not substantially different, these categories were combined. If no clear pattern was observed, the median value or usual limit was used as the cut-off point. As a consequence, two, or rarely three, categories were used for each continuous factor. The same approach was used in the survival analysis as a function of patient group (grafted or non-grafted), strata (as defined in the second step) and their interaction, leading in all cases to two or three different risk groups.[31,32]

SPSS statistical software was used for all statistical analyses (Chicago, IL, USA).

Results

Patients' characteristics

A total of 211 patients from 83 centers, who underwent SCT for PNH between 1978 and 2007, were included in this study. The patients' characteristics are summarized in Table 1. A total of 402 non-transplanted PNH patients diagnosed between 1950 and 2005 had been reported by 92 French centers.[4] The main features of this population, part of a previous report[4] and used for the matching analysis, are detailed in Table 2. Of note none of the patients included in this non-transplanted cohort had received eculizumab.

Table 1. Characteristics of patients and their transplants (n=211).

Characteristics	n/N (%) or median (IQR[a]), N
Gender, female	106/211 (50%)
Age at transplantation, years	30 (23-39)
PNH natural history before SCT, months	20 (7-59), 192
Clone size at transplantation (<3 months before SCT)	56 (32-90), 56
Classification of PNH at transplantation	
Classical PNH	85/191 (45%)
PNH in the setting of another bone marrow disorder	103/191 (54%)[θ]
Indications for SCT[λ,ψ]	
Severe aplastic anemia	118/191 (62%)
Recurrent severe hemolytic crises	64/191 (70%)
Thrombosis[§]	47/191 (25%)
Mesenteric veins	17
Budd Chiari	14
Central nervous system	6
Pulmonary embolism	3
Deep vein thrombosis	2
Myelodysplastic syndrome/acute myeloid leukemia	13/191 (7%)
Donor type	
HLA-identical sibling	136/210 (65%)
Source of stem cells[α]	
Bone marrow	135/210 (64%)
Peripheral blood stem cells	71/210 (34%)
Conditioning regimen	
Cyclophosphamide + busulfan	47/144 (33%)
Cyclophosphamide + total body irradiation (≥ 8 Gray)	22/144 (15%)
Cyclophosphamide + anti-thymocyte globulin	32/144(22%)
Fludarabine-based regimen	42/144 (29%)
GvHD prophylaxis	
Cyclosporine ± methotrexate	154/211 (73%)

[a]IQR: Interquartile range; [θ]Three cases of subclinical PNH; [λ]More than one indication for stem cell transplantation (SCT) was possible; [ψ]Nine patients were transplanted for renal failure and 18 for other reason; [§]Site was lacking for five cases; [α]Four patients received cord blood as the source of stem cells.

Outcomes after transplantation

Engraftment failed in 14 (7%) of the 202 transplanted patients for whom there was documentation on this aspect. Eighty-five patients developed acute GvHD leading to a CIF of grade II-IV acute GvHD of 40% (95%CI 34-47%). Fifty-seven patients developed chronic GvHD (extensive, n=24) leading to a CIF of 29% (95%CI 23% - 36%) at 5 years. Only one patient was documented to have a recurrence of a PNH clone after SCT. After a median (±SE) follow-up time of 61±6 months, 64 patients had died and the 5-year OS probability was 68%±3% (Figure 1A). As shown in *Online Supplementary Table S1*, infections and GvHD were the main causes of death. None of the variables investigated for an association with transplant outcome was a statistically significant predictor of survival (Table 3), except for the indication for SCT with outcome being worse if the indication for SCT was TE (Figure 1B, *P*=0.03). The 5-year OS probability was 54%±7% in the case of TE; 69%±5% in the case of AA without TE and 86%±6% in the case of recurrent hemolytic anemia without TE or AA. Of note, donor type (unrelated *versus* sibling) led to similar results (Figure 1C) (*P*=0.22). Risk factors for survival were also analyzed in patients who were transplanted for TE (n=47) or for AA without TE (n=100) (Table 3). In multivariate analysis, no factor related to survival was identified in patients transplanted for TE. A long delay (>1 year between the diagnosis of AA and transplantation) (*Online Supplementary Figure S1A*) (*P*=0.007), as well as transplantation performed before or in 2002 (*Online Supplementary Figure S1B*) (*P*=0.05) were associated with poor survival in patients transplanted for AA. In this latter group, OS was similar between patients who were transplanted upfront or after immunosuppressive therapy (*data not shown*). Moreover, we did not find any difference according to the stem cell source (*Online Supplementary Figure S2*). However, 15 patients within the 72 patients who received bone marrow as the source of stem cells developed chronic GvHD, compared with nine patients within the 26 patients who received peripheral blood stem cells [5-year CIF of 33% (95%CI 25-41%) and 24% (95%CI 10-38%), respectively; *P*=0.043].

Table 2. Characteristics of non-transplanted patients.

Characteristics	n/N (%) or median (IQR[a]), N
Gender, female	222/402[e] (55%)
Age at diagnosis, years	36 (25-51)
Clone size	30 (15-52), 132
Complications	
Aplastic anemia	59/402
Thrombosis	106/402
Budd Chiari	44
Central nervous system	33
Deep vein thrombosis	31
Pulmonary embolism	7
Myelodysplastic syndrome/acute leukemia	21/402
Treatment	
Immunosuppressive treatment (≥1)[γ]	96/402 (24%)

[a]IQR: Interquartile range; 408 patients were eligible but five did not have follow-up and one was not evaluated for complications; [γ]Immunosuppressive therapy means one course of antithymoglobulin and/or cyclosporine.

Thrombosis: comparison of survival between transplanted and non-transplanted patients

From the 122 patients diagnosed with TE in the SFH cohort, 92 were eligible for the matched-pair analysis, while from the 47 patients who were transplanted for TE in the EBMT population, 42 were eligible. The reasons for exclusion from the matched-pair analysis are detailed in

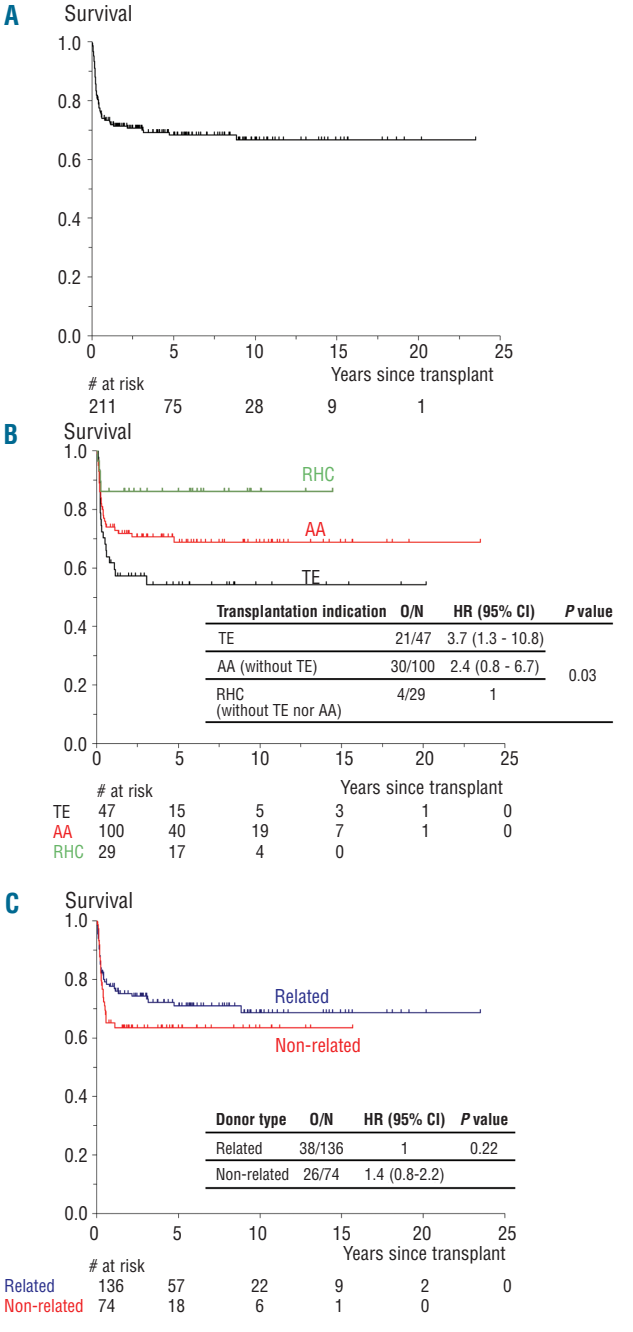

Figure 1. Survival analysis in the overall population (A) and according to transplant indication (B) or donor type (C). (B) Patients transplanted for recurrent hemolytic crisis (RHC) are represented in green, those transplanted for aplastic anemia (AA) in red and those transplanted for thromboembolism (TE) in black. (C) Patients transplanted from a related donor are represented in blue and and patients transplanted from an unrelated donor in red. O/N: number of deaths/number of subjects, HR: hazard ratio, CI: confidence interval.

Online Supplementary Figure S3A. We were able to identify 24 pairs of transplanted and non-transplanted patients. As shown in Figure 2A, a statistically significant difference was observed in OS between the two groups, with better OS for non-transplanted patients (P=0.007). The analysis of patients who could be matched compared to those who could not be matched showed a worse outcome in non-matched patients in the non-transplanted group (*Online Supplementary Figure S4A*), whereas no difference was found in the transplanted group (*Online Supplementary Figure S4B*). We then identified factors associated with OS in transplanted or non-transplanted patients. The year of TE and age at TE were significantly associated with OS.

Table 3. Results of univariate prognostic analyses of overall survival after SCT in patients with PNH.

Covariates	All patients	SCT for thrombosis[#]	SCT for AA* without thrombosis
Period, years			
Before 2002	44/121	12/26	24/66
From 2002 onwards	20/90	8/18	5/33
P	0.15	0.95	0.05
Age at SCT, years			
< 33	33/120	10/23	15/63
≥ 33	31/91	10/21	14/36
P	0.39	0.89	0.09
Sex, recipient			
Male	29/105	8/17	15/59
Female	35/106	12/27	14/40
P	0.33	0.95	0.30
Type of PNH			
Classic	27/85	11/23	9/27
PNH in the setting of another BM disorder	32/103	8/20	20/69
Subclinical PNH	0/3	-	0/3
P	0.59	0.74	0.56
Indications for transplantation[#]			
Recurrent severe hemolytic crises	4/29	-	-
Aplastic anemia	30/100	-	-
Thrombosis	21/47	-	-
P	0.03	-	-
Time from complication to SCT, years			
≤ 1		14/28	6/42
> 1		6/16	23/57
P		0.47	0.007
Source of stem cells			
Bone marrow	37/135	14/30	19/72
Peripheral blood	25/71	5/11	9/26
Cord blood	2/4	1/3	1/1
P[§]	0.18	0.79	0.40
Type of donor			
Sibling	38/136	11/26	18/68
Unrelated	26/74	9/18	11/31
P	0.22	0.39	0.48
Conditioning regimen			
MAC[α]	23/70	7/18	11/30
RIC	15/42	4/10	4/13
Cyclophosphamide and ATG	4/32	2/5	2/19
P	0.07	0.96	0.18

*Results are by number of deaths / total number of patients; [#]patients with thrombosis, *severe aplastic anemia without thrombosis; [§]cord blood excluded ; MAC: standard conditioning regimen (oral busulfan ≥ 8 mg/Kg or intravenous busilvex ≥ 6.4 mg/Kg or total body irradiation ≥ 8 Grays); RIC: reduced intensity conditioning regimen; ATG: anti-thymocyte globulin. course of anti thymocyte globulin and/or cyclosporine.*

We then selected patients with a date of TE after 1990 (there were very few patients in the transplanted group with a TE before this date) and who were less than 60 years old at the time of TE (there were no patients in the transplanted group who were 60 years of age or more at TE). After these selections (leading to 39 and 41 patients in the non-transplanted and transplanted groups, respectively) younger age at TE and shorter interval between PNH diagnosis and TE were significantly associated with better OS, leading to two prognostic strata: stratum A (age <30 years and time interval ≥3 months, or time interval < 3 months) and stratum B (age ≥30 years and time interval ≥ 3 months). Results of the analysis stratified on strata A/B are shown in *Online Supplementary Figure S5A* (stratum A) and *Online Supplementary Figure S5B* (Stratum B). The results of the analysis adjusted on stratum are shown in Figure 2B: stratum A non-transplanted (HR = 1), patients transplanted [HR = 4.1 (95%CI 1.2-13.8)] and stratum B non-transplanted [HR = 5.8 (95%CI 1.2-28.9)].

Aplastic anemia without thrombotic events: comparison of survival between transplanted and non-transplanted patients

From the 141 patients diagnosed with aplastic anemia in the SFH cohort, 99 were eligible for the matched-pair analysis while from the 119 patients who were transplanted, 100 were eligible. The reasons for exclusion from the matched-pair analysis, particularly the concomitant diagnosis of TE, are detailed in *Online Supplementary Figure S3B*. We identified 30 pairs of transplanted and non-transplanted patients eligible for comparison. As shown in Figure 3, there was a borderline, not statistically significant difference in favor of immunosuppressive therapy over SCT (P=0.06). The OS analyses in matched *versus* non-matched patients in the non-transplanted group as well as in the transplanted group were not statistically different (*Online Supplementary Figure S6A and S6B*, respectively). We then identified factors associated with OS in the transplanted and in the non-transplanted patients. Year of AA and age at AA, as well as the time interval between PNH diagnosis and the complication of AA were significantly associated with OS. We then selected patients whose AA occurred after 1970 (no patient in the transplanted group developed AA before this date) and were aged 10 to 50 years old, (there were no patients in the transplanted group more than 50 years old and only one less than 10 years of age in the non-transplanted group). After these selections, leading to 69 and 97 patients in the non-transplanted and transplanted groups, respectively, age at AA, date of AA and the time interval between PNH diagnosis and AA, were significantly associated with better OS, leading to too many strata (n=12). Further selection led to one or no deaths in the non-transplanted group. For these two reasons, global matching was not feasible for AA-PNH patients.

Discussion

In this large retrospective study, we determined the characteristics and OS of 211 patients transplanted for PNH in 83 EBMT centers from 1978 to 2007. The indication for SCT was the only significant predictor of survival, with patients transplanted for TE having the worst outcome. Next, we conducted an analysis with a cohort of

	O/N	HR (95% CI)	*P* value
Non-transplanted	3/24	1	0.007
Transplanted	12/24	10.0 (1.3 - 78.7)	

	O/N	HR (95% CI)	*P* value
Non-transplanted stratum A	3/33	1	0.03
Transplanted	18/41	4.1 (1.2 - 13.8)	
Non-transplanted stratum B	3/6	5.8 (1.2 - 28.9)	

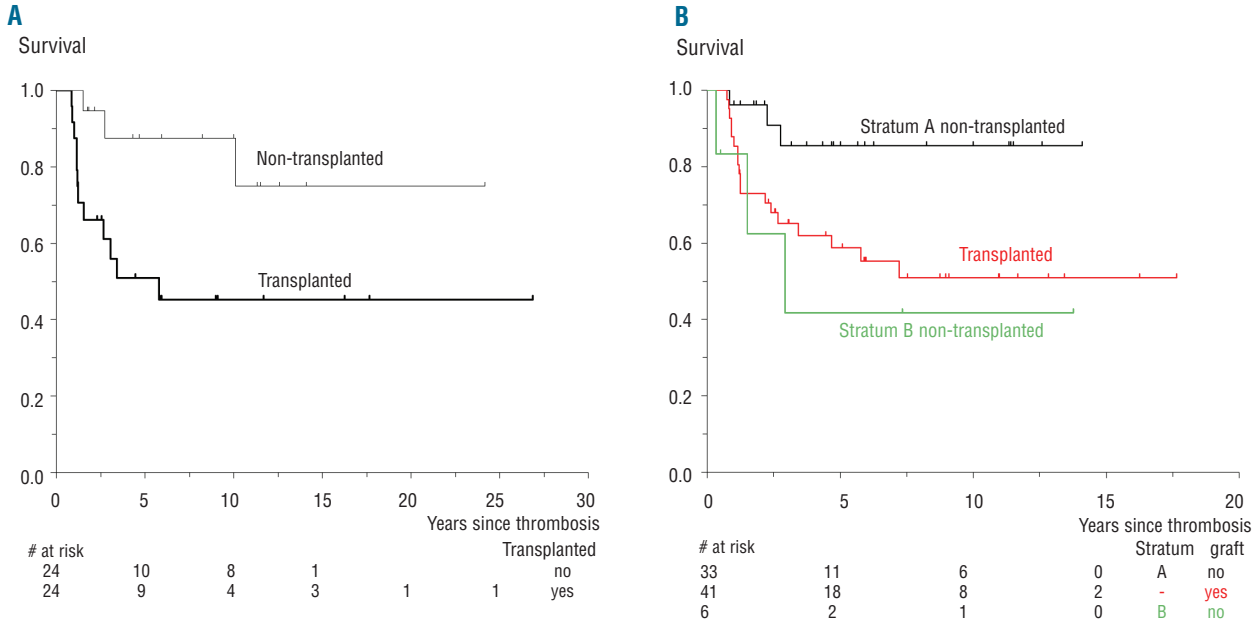

Figure 2. Matching between transplanted and non-transplanted patients in the case of thrombotic events. Survival analysis in 24 matched pairs of transplanted (bold line) *versus* non-transplanted patients (thin line) is represented in (A). Global matching is represented in (B). Non-transplanted patients of stratum A (age <30 years and delay ≥ 3 months, or delay < 3 months) are represented in black, transplanted patients are represented in red (all strata) and non-transplanted patients of stratum B are represented in green (age ≥ 30 years and delay ≥ 3 months). O/N: number of deaths/number of subjects, HR: hazard ratio, CI: confidence interval

402 non-transplanted PNH patients in 92 French centers. The comparison between matched transplanted and non-transplanted patients in the case of TE showed a worse OS for the transplanted patients.

The clinical course of PNH is highly variable. The disease can persist for many years with manageable symptoms, or patients may even recover spontaneously.[33,34] In other cases, the disease course is aggressive with life-threatening complications including TE, bone marrow failure, or myelodysplastic syndromes.[4,33] Allogeneic SCT is able to eradicate the PNH clone in patients with classical PNH and AA-PNH; however, it is associated with significant morbidity and mortality.[1,22] In recent years, the introduction of eculizumab, a humanized monoclonal antibody directed against the terminal complement protein C5, has had a major impact on the management of PNH. Eculizumab is highly effective in reducing intravascular hemolysis and seems to reduce the risk of thrombosis markedly.[5,6,35] However, roughly 50% of patients will require transfusions under eculizumab[6] and it does not improve bone marrow failure in the setting of the AA-PNH syndrome. It increases the risk of meningococcal sepsis and the very long-term survival with eculizumab is not yet well known. Moreover, eculizumab is expensive (around € 300,000 per year for each patient), does not eradicate the PNH clone, and must be given lifelong. Identifying patients with PNH who may benefit from SCT

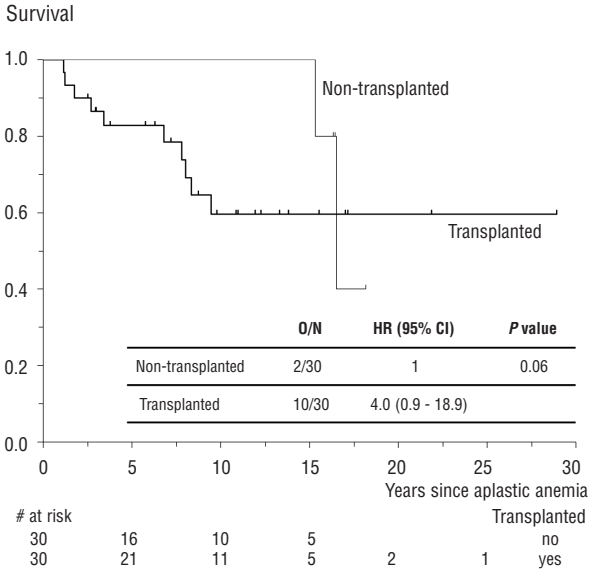

Figure 3. One to one matching between transplanted and non-transplanted patients in the case of aplastic anemia. Survival analysis of 30 matched pairs of transplanted (bold line) *versus* non-transplanted patients (thin line) is represented. O/N: number of deaths/number of subjects, HR: hazard ratio, CI: confidence interval.

is, therefore, an important, albeit particularly difficult challenge. Several transplant-related issues could not be addressed in previous studies because of the small numbers of patients investigated. The most important issues, when and in whom a transplant should be performed, have never been addressed mainly because a prospective trial comparing alternative treatment strategies is impossible in such a rare disease.

In this study, we reviewed the outcome of 211 patients, which represents the largest study on PNH and SCT ever done to date. There are few reports on the use of SCT for PNH, and nearly all of them are based on small numbers of patients except two registry studies: one from the International Bone Marrow Transplant Registry (IBMTR) that included 57 patients[23] and one from an Italian group on behalf of the *Gruppo Italiano Trapianto Midollo Osseo* (GITMO), including 26 patients.[36] The main indications for transplantation in these and in our series were AA-PNH in about 50% of the cases. In our series, graft failure occurred in 6% of patients, a rate similar to that recently published by the GITMO.[36] The place of a reduced intensity conditioning regimen in PNH, especially for patients with moderate organ dysfunction who may not tolerate a myeloablative regimen, is still unknown. In our study, 42 patients received a fludarabine-based reduced intensity conditioning regimen and showed no difference in terms of treatment-related mortality or OS (*data not shown*). However, the retrospective setting of our study as well as the heterogeneity of the conditioning regimens precluded further analyses of the role of conditioning in the present series. Acute and chronic GvHD occurred in roughly one third of our patients, which is not different to rates found in previous studies. With a median follow-up of 61 months, the OS rate was 68%, while 32% of the patients died of a cause related to treatment. Infectious complications were the primary cause of mortality in our series. This complication has been underestimated in previous smaller series, but is not unexpected considering the number of aplastic patients and the general susceptibility to infection in PNH.[4] The second cause of death was, as expected, GvHD.[1] One of the main findings of the present study is the strong impact of indication for transplantation on OS and, in particular, the poor outcome of patients being transplanted for TE. More than one third of our patients were transplanted with stem cells from unrelated donors and, surprisingly, had an OS rate comparable to those transplanted with a graft from an HLA-identical sibling donor. Unrelated transplantation for AA has improved significantly over the past 15 years,[37] mainly due to better HLA matching, which may partly explain similar outcomes in the present study.

The most challenging problem, however, is to identify those PNH patients who would benefit from SCT. To try to resolve this problem, we performed comparative analyses with matched cohorts of PNH patients who did or who did not undergo SCT. The main problem to be solved, when comparing two cohorts of patients receiving different treatments, is the comparability of the two cohorts for all criteria related to outcome. Comparability cannot be guaranteed in the absence of treatment allocation through randomization, a challenging task in the setting of an orphan disease. Propensity score matching[38] could not be used here because although the same data were available for both cohorts, the data were not collected at the same time (at diagnosis for non-transplanted patients, and at the time of SCT for transplanted patients). Since the only factor related to OS was the indication for SCT in the transplanted group, and because the occurrence of a complication was the most important prognostic factor related to survival in the non-transplanted group, we compared patients experiencing the same complication (i.e. TE and AA without TE) in the pre-eculizumab era. The individual matching comparison can be discussed. Indeed, in the non-transplanted group, patients selected for comparison had a better OS than those who were not selected, with unexpectedly good outcome for patients with TE and PNH. However, the global comparison did not change the results, clearly not in favor of transplantation for TE. These results suggest that SCT can no longer be considered a standard of care for PNH patients with TE.

The situation was different in patients with AA. In these patients, a small PNH clone is frequently detected by flow cytometry in the absence of hemolysis and in the presence of a hypocellular bone marrow.[39] Patients with AA-PNH syndrome are treated in the same was as patients with AA, regardless of the presence of a PNH clone.[10,40,41] In our study, the best results were obtained in patients with severe AA-PNH syndrome who were transplanted with a graft from an HLA-identical sibling after year 2002. A first course of immunosuppressive treatment with antithymocyte globulin plus cyclosporine before SCT gave similar results but there were few patients managed in this way (n=16). We also found the importance of bone marrow as a source of stem cells in this situation.[4,42] While OS was not different between patients grafted with stem cells from the two difference sources, a higher rate of chronic GvHD was observed in peripheral blood stem cell recipients. Although this study reports data from the largest cohort of patients undergoing SCT for PNH so far and is quite unique in its aim to compare outcomes with non-transplanted patients, it has limitations inherent to retrospective analyses comparing different cohorts.

In conclusion, given our present results, and the efficacy of eculizumab, the current indications for SCT should be challenged. SCT can no longer be considered as a standard of care of PNH patients with TE when eculizumab is available. Regarding the good results of SCT in the case of recurrent hemolytic crises, SCT can be a valuable option for patients living in countries which cannot afford eculizumab, regardless of the type of donor. AA-PNH patients still seem to be appropriate candidates for SCT if they have severe disease. In the absence of an HLA-identical sibling donor, the current standard, first-line treatment for AA-PNH syndrome is immunosuppressive therapy.

Authorship and Disclosures

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References

1. Parker C, Omine M, Richards S, Nishimura J, Bessler M, Ware R, et al. Diagnosis and management of paroxysmal nocturnal hemoglobinuria. Blood. 2005;106(12):3699-709.
2. Dameshek W. Riddle: what do aplastic anemia, paroxysmal nocturnal hemoglobinuria (PNH) and "hypoplastic" leukemia have in common? Blood. 1967;30(2):251-4.
3. Socie G, Mary JY, de Gramont A, Rio B, Leporrier M, Rose C, et al. Paroxysmal nocturnal haemoglobinuria: long-term follow-up and prognostic factors. French Society of Haematology. Lancet. 1996;348(9027):573-7.
4. Peffault de Latour R, Mary JY, Salanoubat C, Terriou L, Etienne G, Mohty M, et al. Paroxysmal nocturnal hemoglobinuria: natural history of disease subcategories. Blood. 2008;112(8):3099-106.
5. Hillmen P, Hall C, Marsh JC, Elebute M, Bombara MP, Petro BE, et al. Effect of eculizumab on hemolysis and transfusion requirements in patients with paroxysmal nocturnal hemoglobinuria. N Engl J Med. 2004;350(6):552-9.
6. Hillmen P, Young NS, Schubert J, Brodsky RA, Socie G, Muus P, et al. The complement inhibitor eculizumab in paroxysmal nocturnal hemoglobinuria. N Engl J Med. 2006;355(12):1233-43.
7. Helley D, de Latour RP, Porcher R, Rodrigues CA, Galy-Fauroux I, Matheron J, et al. Evaluation of hemostasis and endothelial function in patients with paroxysmal nocturnal hemoglobinuria receiving eculizumab. Haematologica. 2010;95(4):574-81.
8. Hillmen P, Muus P, Duhrsen U, Risitano AM, Schubert J, Luzzatto L, et al. Effect of the complement inhibitor eculizumab on thromboembolism in patients with paroxysmal nocturnal hemoglobinuria. Blood. 2007;110(12):4123-8.
9. Kelly RJ, Hill A, Arnold LM, Brooksbank GL, Richards SJ, Cullen M, et al. Long-term treatment with eculizumab in paroxysmal nocturnal hemoglobinuria: sustained efficacy and improved survival. Blood. 2011;117(25):6786-92.
10. Young NS, Scheinberg P, Calado RT. Aplastic anemia. Curr Opin Hematol. 2008;15(3):162-8.
11. Takahashi Y, McCoy JP, Jr., Carvallo C, Rivera C, Igarashi T, Srinivasan R, et al. In vitro and in vivo evidence of PNH cell sensitivity to immune attack after nonmyeloablative allogeneic hematopoietic cell transplantation. Blood. 2004;103(4):1383-90.
12. Storb R, Evans RS, Thomas ED, Buckner CD, Clift RA, Fefer A, et al. Paroxysmal nocturnal haemoglobinuria and refractory marrow failure treated by marrow transplantation. Br J Haematol. 1973;24(6):743-50.
13. Antin JH, Ginsburg D, Smith BR, Nathan DG, Orkin SH, Rappeport JM. Bone marrow transplantation for paroxysmal nocturnal hemoglobinuria: eradication of the PNH clone and documentation of complete lymphohematopoietic engraftment. Blood. 1985;66(6):1247-50.
14. Bemba M, Guardiola P, Garderet L, Devergie A, Ribaud P, Esperou H, et al. Bone marrow transplantation for paroxysmal nocturnal haemoglobinuria. Br J Haematol. 1999;105(2):366-8.
15. Endo M, Beatty PG, Vreeke TM, Wittwer CT, Singh SP, Parker CJ. Syngeneic bone marrow transplantation without conditioning in a patient with paroxysmal nocturnal hemoglobinuria: in vivo evidence that the mutant stem cells have a survival advantage. Blood. 1996;88(2):742-50.
16. Fefer A, Freeman H, Storb R, Hill J, Singer J, Edwards A, et al. Paroxysmal nocturnal hemoglobinuria and marrow failure treated by infusion of marrow from an identical twin. Ann Intern Med. 1976;84(6):692-5.
17. Hegenbart U, Niederwieser D, Forman S, Holler E, Leiblein S, Johnston L, et al. Hematopoietic cell transplantation from related and unrelated donors after minimal conditioning as a curative treatment modality for severe paroxysmal nocturnal hemoglobinuria. Biol Blood Marrow Transplant. 2003;9(11):689-97.
18. Lee JL, Lee JH, Lee JH, Choi SJ, Kim S, Seol M, et al. Allogeneic hematopoietic cell transplantation for paroxysmal nocturnal hemoglobinuria. Eur J Haematol. 2003;71(2):114-8.
19. Raiola AM, Van Lint MT, Lamparelli T, Gualandi F, Benvenuto F, Figari O, et al. Bone marrow transplantation for paroxysmal nocturnal hemoglobinuria. Haematologica. 2000;85(1):59-62.
20. Suenaga K, Kanda Y, Niiya H, Nakai K, Saito T, Saito A, et al. Successful application of nonmyeloablative transplantation for paroxysmal nocturnal hemoglobinuria. Exp Hematol. 2001;29(5):639-42.
21. Szer J, Deeg HJ, Witherspoon RP, Fefer A, Buckner CD, Thomas ED, et al. Long-term survival after marrow transplantation for paroxysmal nocturnal hemoglobinuria with aplastic anemia. Ann Intern Med. 1984;101(2):193-5.
22. Matos-Fernandez NA, Abou Mourad YR, Caceres W, Kharfan-Dabaja MA. Current status of allogeneic hematopoietic stem cell transplantation for paroxysmal nocturnal hemoglobinuria. Biol Blood Marrow Transplant. 2009;15(6):656-61.
23. Saso R, Marsh J, Cevreska L, Szer J, Gale RP, Rowlings PA, et al. Bone marrow transplants for paroxysmal nocturnal haemoglobinuria. Br J Haematol. 1999;104(2):392-6.
24. Rosse WF. Dr Ham's test revisited. Blood. 1991;78(3):547-50.
25. Hall SE, Rosse WF. The use of monoclonal antibodies and flow cytometry in the diagnosis of paroxysmal nocturnal hemoglobinuria. Blood. 1996;87(12):5332-40.
26. Przepiorka D, Weisdorf D, Martin P, Klingemann HG, Beatty P, Hows J, et al. 1994 Consensus Conference on Acute GVHD Grading. Bone Marrow Transplant. 1995;15(6):825-8.
27. Fine J, Gray R. A proportional hazards models for sub-distribution of a competing risk. J Am Stat Ass. 1999;94:496-509.
28. Kaplan EL, Meier P. Non parametric estimation for incomplete observations. J Am Stat Ass. 1958;53:457-81.
29. Mantel N. Evaluation of survival data and two new rank order statistics arising in its consideration. Cancer Chemother Rep. 1966;50(3):163-70.
30. Cox DR. Regression models and life-tables (with discussions), series B. J Roy Statist Soc. 1972;34:184-192.
31. Peto R, Pike MC, Armitage P, Breslow NE, Cox DR, Howard SV, et al. Design and analysis of randomized clinical trials requiring prolonged observation of each patient. II. analysis and examples. Br J Cancer. 1977;35(1):1-39.
32. Byar DP. Identification of prognosis factors. In: Buyse ME, Staquet MJ, Sylvester RJ, eds. Cancer clinical trials, methods and practice. Oxford: Oxford Medical Publications. 1988:423-43.
33. Hillmen P, Lewis SM, Bessler M, Luzzatto L, Dacie JV. Natural history of paroxysmal nocturnal hemoglobinuria. N Engl J Med. 1995;333(19):1253-8.
34. Rosse WF, Nishimura J. Clinical manifestations of paroxysmal nocturnal hemoglobinuria: present state and future problems. Int J Hematol. 2003;77(2):113-20.
35. Brodsky RA, Young NS, Antonioli E, Risitano AM, Schrezenmeier H, Schubert J, et al. Multicenter phase 3 study of the complement inhibitor eculizumab for the treatment of patients with paroxysmal nocturnal hemoglobinuria. Blood. 2008;111(4):1840-7.
36. Santarone S, Bacigalupo A, Risitano AM, Tagliaferri E, Di Bartolomeo E, Iori AP, et al. Hematopoietic stem cell transplantation for paroxysmal nocturnal hemoglobinuria: long-term results of a retrospective study on behalf of the Gruppo Italiano Trapianto Midollo Osseo (GITMO). Haematologica. 2010;95(6):983-8.
37. Maury S, Balere-Appert ML, Chir Z, Boiron JM, Galambrun C, Yakouben K, et al. Unrelated stem cell transplantation for severe acquired aplastic anemia: improved outcome in the era of high-resolution HLA matching between donor and recipient. Haematologica. 2007;92(5):589-96.
38. Rosenbaum PR. The central role of the propensity score in observational studies for causal effects. Biometrika. 1983 1983;70:41-55.
39. Dunn DE, Tanawattanacharoen P, Boccuni P, Nagakura S, Green SW, Kirby MR, et al. Paroxysmal nocturnal hemoglobinuria cells in patients with bone marrow failure syndromes. Ann Intern Med. 1999;131(6):401-8.
40. Marsh JC, Ball SE, Cavenagh J, Darbyshire P, Dokal I, Gordon-Smith EC, et al. Guidelines for the diagnosis and management of aplastic anaemia. Br J Haematol. 2009;147(1):43-70.
41. Kojima S, Nakao S, Young N, Bacigalupo A, Gerard G, Hirano N, et al. The Third Consensus Conference on the treatment of aplastic anemia. Int J Hematol. 2011;93(6):832-7.
42. Schrezenmeier H, Passweg JR, Marsh JC, Bacigalupo A, Bredeson CN, Bullorsky E, et al. Worse outcome and more chronic GVHD with peripheral blood progenitor cells than bone marrow in HLA-matched sibling donor transplants for young patients with severe acquired aplastic anemia. Blood. 2007;110(4):1397-400.
43. Eapen M, Le Rademacher J, Antin JH, Champlin RE, Carreras J, Fay J, et al. Effect of stem cell source on outcomes after unrelated donor transplantation in severe aplastic anemia. Blood. 2011;118(9):2618-21.