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Letter
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Allogeneic Hematopoietic Cell Transplantation in Patients With Aplastic Anemia and Paroxysmal Nocturnal Hemoglobinuria Clones: Time for a Change

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Dear Editor, Acquired severe aplastic anemia (SAA) remains a diagnostic and therapeutic challenge, despite advances in the field that have led to improved survival rates.[1] Allogeneic hematopoietic cell transplantation (alloHCT) is a cornerstone in the therapeutic algorithm of the disease even in patients with coexisting paroxysmal nocturnal hemoglobinuria (PNH) clones. Nevertheless, literature on patients with SAA and PNH clones post alloHCT is scarce.

Complement inhibition with eculizumab has shown effectiveness in patients with PNH and high disease activity, with or without aplastic anemia.[2] However, the role of eculizumab in the transplant setting has not been clarified yet. Recently, DeZern et al reported successful outcomes with eculizumab bridging before alloHCT in 8 SAA patients.[3] Two recent studies have also explored outcomes of patients with PNH clones in the age of eculizumab.[4,5] Although both studies present the potential benefits of eculizumab post alloHCT in 8 and 2 patients respectively, there is no clear comparison with a historical control group that did not receive eculizumab.[4,5] This comparison would clarify the role of complement inhibition, given that alloHCT mortality in SAA patients with PNH clones has been reported at approximately 30%.[6]

Given the renewed interest in the field, we conducted a retrospective analysis of alloHCT outcomes in patients transplanted for SAA with or without PNH clones.

We enrolled consecutive patients that underwent alloHCT for SAA in our JACIE-accredited center (1992–2018). Standard operating procedures of our center were followed as previously described.[7,8] All patients were evaluated for a presence of a PNH clone using our standardized flow cytometry protocol based on FLAER (fluorescent aerolysin) detection at diagnosis and before transplant.[9] The conditioning regimen consisted of Cyclophosphamide (50 mg/kg /day × 4 days) and Antithymocyte Globulin (Thymoglobuline 2.5 mg/kg/day×3 days). In patients sensitized with multiple transfusions[10] the conditioning regimen was modified to Cyclophosphamide (50 mg/kg /day × 4 days), Fludarabine (30 mg/m2/day × 4 days), ATG 7.5 mg/kg and 10 mg/kg for sibling and unrelated donors respectively, as previously described.[11] Patients received GVHD prophylaxis with Methotrexate and Cyclosporine. Cyclosporine was slowly tapered and stopped between 9 and 12 months post-transplant with a careful follow-up of blood counts. STR (short tandem repeat) fragment analysis was performed regularly (on day + 14, + 30, +60, + 90) in unfractionated bone marrow for chimerism evaluation. Complete donor chimerism was defined as donor chimerism ≥99%.[12] Regarding supportive care, patients were admitted to neutropenic isolation rooms with HEPA filters. Prophylaxis for Pneumocystis jiroveci, herpes simplex, and Candida spp was administered. Patients underwent cytomegalovirus and Epstein-Barr surveillance using peripheral blood molecular assays.[13] Patients with PNH clones received prophylactic anticoagulation as standard practice.

This study was approved by the institutional review board and ethics committee of G. Papanicolaou Hospital. All patients gave written informed consent. The study was conducted in compliance with the Helsinki Declaration.

Data were analyzed with the SPSS 22.0 (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp) statistical program. Statistical analysis included pretransplant factors: time from diagnosis to transplant, pre-transplant transfusions, pre-transplant lines of treatment, age, gender, hemoglobin and neutrophils at transplant, PNH clone, ECOG performance status; transplant factors: donor type (sibling/unrelated), graft source (bone marrow/peripheral), HLA matching, conditioning (Cyclophosphamide/ATG globulin with/without fludarabine); post-transplant factors: neutrophil and platelet engraftment, severe acute (grade 2-4) and extensive chronic GVHD, transplant-associated thrombotic

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Table 1

Summary of patients' characteristics (n = 24)

Age (years)	20 (9–46)
Gender (male:female, n)	9:15
Refractory disease at transplant (n)	14
Previous lines of immunosuppression	2 (1–4)
Pre-transplant RBC transfusions	22 (6–125)
Pre-transplant PLT transfusions	28 (15–680)
Grafts (bone marrow:PBSCs)	19:5
Donors (sibling:unrelated)	15:9
Fludarabine-based conditioning	13
Patients with PNH clones (n)	3
Size of PNH clones in neutrophils (median, range)	68, 61–82

Continuous variables are presented as median (range). PBSCs: peripheral blood stem cells. RBC: red blood cells. PLT: platelets.

microangiopathy (TA-TMA), post-transplant lymphoproliferative disease (PTLD), infections, late complications, relapse, and survival. Chi-square test, Student's *t* test or Mann–Whitney test were used to compare the above-mentioned variables. Overall survival (OS) probability was calculated with Kaplan–Meier curves; while Cox proportional hazards were used for multivariable analysis of OS. Cumulative incidence of competing events analysis was performed using the EZR software (http://www.jichi.ac.jp/saitama-sct/SaitamaHP.files/statmed.html).[14] Statistical significance was assessed by the Gray test and Fine and Gray regression modeling. Variables with $p \leq 0.05$ in the univariate analysis were selected for the multivariate model. Significance level was 0.05 and two-tailed.

In total, 24 patients were studied. Severe AA was diagnosed in 18 and very severe in 6 patients. PNH clones were detected only in 3 patients that did not present with signs of hemolysis or thrombosis. PNH clones in these patients were equal or larger than 60% in neutrophils. Table 1 summarizes basic patients' characteristics. Bone marrow grafts and sibling donors were preferred when available.

Engraftment was evident at day 15 post-transplant (range 12–21) for neutrophils and 42 (17–121) for platelets. Complete donor chimerism was achieved in all patients. There was no graft rejection or failure. Patients were followed-up for a large period of 59 months (0.9–245), with the follow-up among survivors reaching 114 (13–245) months. Three patients developed TA-TMA, that resolved in 2 out of 3 patients following cyclosporine cessation. Two patients presented with PTLD. The first patient had central nervous system involvement with a fatal outcome, whereas the second patients had a successful resolution following Rituximab administration.

Grade 2–4 acute GVHD showed a cumulative incidence (CI) of 22.2%. In the multivariate model, peripheral grafts and TA-TMA were independent predictors of acute GVHD CI ($p < 0.001$). Chronic GVHD showed a 10-year CI of 31.6% and was independently predicted by hemoglobin at transplant ($p = 0.007$), HLA mismatch ($p < 0.001$), PNH clones ($p < 0.001$) and TA-TMA ($p < 0.001$). Lastly, 10-year OS reached 70.6% and was independently associated with PNH clones ($p = 0.034$, Fig. 1). We

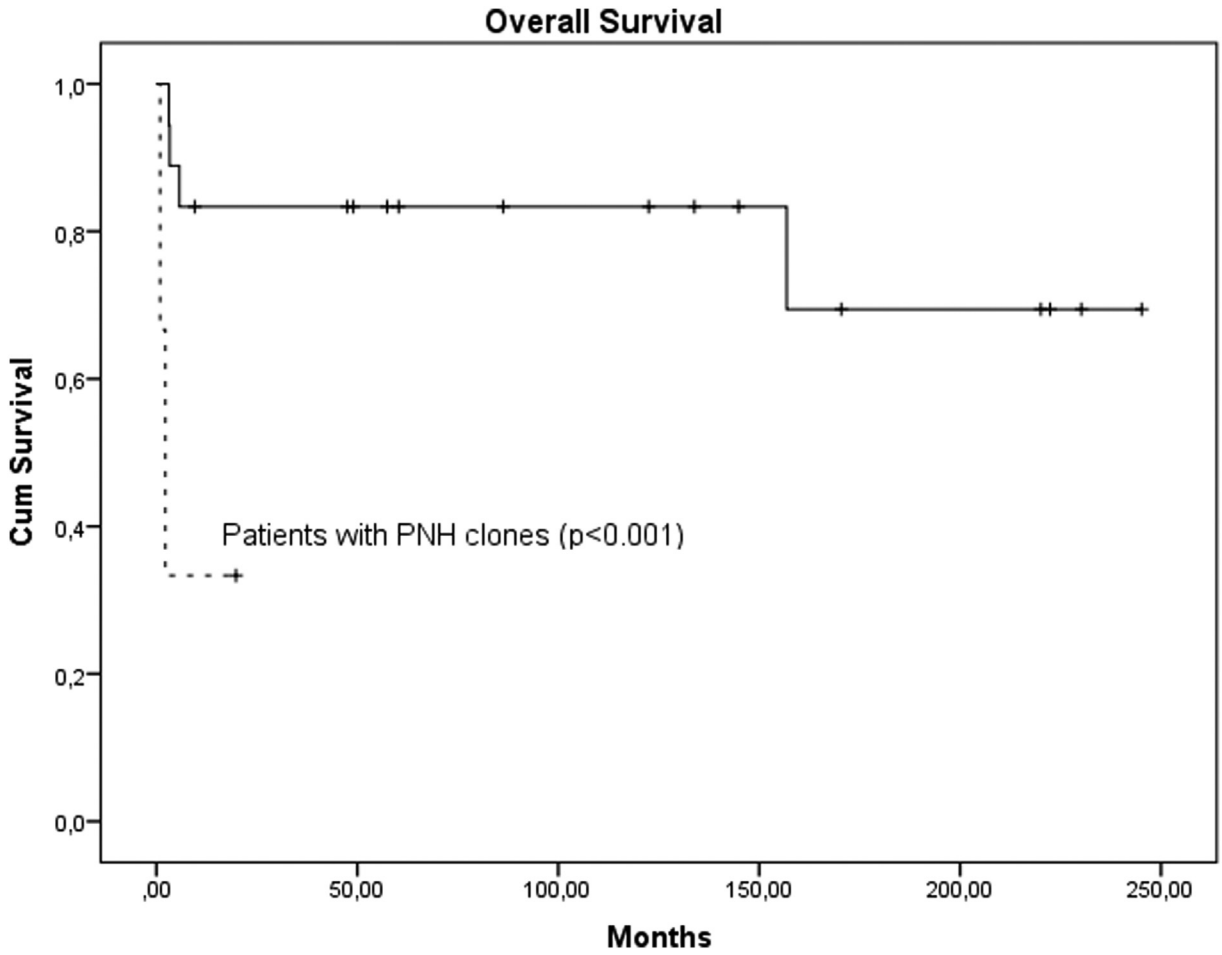

Figure 1. Overall survival is significantly lower in patients with PNH clones ($p < 0.001$). PNH: paroxysmal nocturnal hemoglobinuria.

did not observe secondary malignancies or fatal long-term complications. Treatment-related mortality associated with GVHD and infectious complications was the cause of death in patients without PNH clones.

All 3 PNH patients achieved full donor chimerism. However, 2 patients succumbed to thrombotic complications associated with severe acute GVHD and multiple organ failure during the early post-transplant period. Eculizumab was not available during the period of these complications.

Our study highlights the presence of a PNH clone as a negative predictor of survival post alloHCT in SAA patients, independently of other factors studied. Despite that concomitant diagnosis of PNH and SAA is common, only a few recent reports of SAA outcomes have taken into account the presence of PNH clones.[15]

PNH has been traditionally considered a contributor to worse outcomes after alloHCT due to the heterogeneity of clinical presentations and severe signs of hemolysis and thrombocytopenia.[16] It should be noted that PNH clones detected in our patients were similar to those observed in clinical PNH patients. Thrombosis is the major cause of death in PNH patients.[17] Despite the multifactorial nature of thrombosis in PNH, complement inhibition seems to play a crucial role by blocking this vicious cycle.[17] However, little is known about patients post alloHCT. In this setting, early prediction of cardiovascular or thrombotic risk is not yet feasible.[18]

In the group of SAA patients without a PNH clone, our findings were comparable to those recently reported by the Aplastic Anemia Working Party of the European Group for Blood and Marrow Transplantation with the use of sibling or unrelated donors.[19] The majority of recent previous reports has documented a risk of graft rejection/failure ranging from 3% to 33%.[15] In our cohort, there was no graft rejection/failure. The use of fludarabine in the conditioning regimen along with ATG might have contributed to this result.[20]

Our study is limited by its retrospective nature, the rather small number of participants and the single-center experience of a long period of time during which advances in diagnostics and therapeutics may have led to improved outcomes. In addition, our study was performed with both sibling and unrelated donors, since expansion of the donor pool using alternative donors remains currently under consideration as an alternative option for those patients.[21,22] It should be noted however that this study was conducted according to standard operating procedures with a long-term follow-up.

In conclusion, the presence of a PNH clone worsens the outcomes of alloHCT in AA patients. Our report is in line with important findings of recent studies on eculizumab providing a control population of AA patients with and without PNH clones.[4,5] Despite the rather small population of relevant studies, these findings support the notion that in the era of effective complement inhibition, novel approaches are may improve morbidity and mortality in patients with PNH clones. Future larger studies are warranted to determine the indications of complement inhibition before and after alloHCT.

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