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Current Status of Stem Cell Transplantation in Paroxysmal Nocturnal Hemoglobinuria

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Abstract:

Paroxysmal nocturnal hemoglobinuria (PNH) is a clonal, acquired hematopoietic stem cell disorder, which is caused by activation of the complement system leading to life-threatening manifestations such as hemolysis, thrombosis, and marrow failure. Eculizumab is a complement inhibitor of C5, which acts by blocking complement-mediated hemolysis. It needs to be administered lifelong to the patient; hence, there are major financial implications. This drug is easily available in the Western countries; however, in low resource countries, where its availability is limited, hematopoietic stem cell transplantation (HSCT) still remains the main modality for achieving cure in PNH. PNH being a rare disease, large prospective studies and guidelines are scarce. To choose the ideal candidate for transplant is the real challenge. This article aims to review the trends in HSCT for PNH, such as the use of reduced-intensity conditioning to attain the graft versus PNH effect or the use of haploidentical donors. In the era of complement inhibitor therapy, the role of transplantation still needs to be explored.

Keywords:

Paroxysmal nocturnal hemoglobinuria, stem cell transplantation, eculizumab

Introduction

Paroxysmal nocturnal hemoglobinuria (PNH) is a clonal hematological disorder due to the proliferation of abnormal hematopoietic stem cells, carrying a unique mutation in the Phosphatidylinositol N-acetylglucosaminyl transferase subunit A (PIG-A) gene. PNH can present as a classical hemolytic variety or as bone marrow failure (Aplastic anemia/AA). Patients with the classical form of PNH have elevated reticulocyte count, lactate dehydrogenase , and normocellular to hypercellular bone marrow. They may also present with either excessive hemolysis or thrombosis. Small populations of PNH cells are also seen in patients with acquired AA or Myelodysplastic syndromes (MDS). Patients with overlap between AA and PNH (PNH/AA); present with cytopenias, reticulocytopenia, and hypocellular bone marrow.[1]

Hematopoietic stem cell transplantation (HSCT) is the only proven modality of the cure for PNH,[2,3] it is however, associated with significant morbidity and mortality. Eculizumab (anti-C5 antibody) was approved in 2007, after which the only indications for transplant in PNH include bone marrow failure, refractory transfusion-dependent hemolytic anemia, or recurring thromboembolic complications, not responding to eculizumab. It needs to be administered lifelong to the patient; hence, it has major cost implications. In countries where eculizumab availability is still limited, HSCT still remains the only means to achieve cure. Large prospective studies or guidelines on HSCT for PNH are scarce. This article aims to review the published data and current standards of HSCT in PNH.

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Transplant for Paroxysmal Nocturnal Hemoglobinuria: Landmark Studies Till Date

The first documented case of PNH transplant was in the 1970s. The transplant was done without conditioning; the donor was a twin who later relapsed due to a mutation in the PIG-A gene. HSCT from twin donors using conditioning regimen proved successful with a low incidence of relapse.[4,5] Since then, many isolated case reports and single-center experiences have been cited in literature, but large prospective studies are very few and far in between.[6-36]

The center for international blood and marrow transplant registry (CIBMTR) data showed that the 2-year probability of survival in 48 recipients of human leukocyte antigen (HLA)-identical sibling transplants was 56% (95% confidence interval: 49%–63%), at a median follow-up period of 44 months. Engraftment failure occurred in 7 patients and death due to infections occurred in 3 patients. The incidence of acute graft-versus-host disease (GVHD) (grade > II or more) and chronic GVHD was 34%, and 33% respectively.[23]

A recent retrospective study from the Gruppo Italiano Trapianto Midollo Osseo (GITMO) showed a 57% 10-year survival of 26 transplanted PNH patients (4 AA/PNH). Rates of acute and chronic GVHD were 42% and 50%, respectively. The treatment-related mortality (TRM) was higher in reduced-intensity conditioning (RIC) transplants (63%, n = 11) as compared to myeloablative transplants (26%, n = 15). The authors attributed this difference to the higher number of sick patients receiving RIC. Many unrelated transplants also received RIC.[31]

The European Group for Blood and Marrow Transplantation (EBMT) published its data of 211 patients transplanted for PNH at 83 EBMT centers. The indications for HSCT were AA (n = 100, 48%), recurrent hemolytic crisis (n = 64, 30%), and thrombosis (n = 47, 22%). Engraftment failure was seen in 14 (7%) patients. Infections and GVHD were the main cause of death. Patients undergoing HSCT for recurrent thromboembolism had worse outcomes (P = 0.03).[32]

The French PNH registry and the registry from the *Société Francophone de Greffe de Moelle et de Thérapie Cellulaire*, reported the results of HSCT for PNH in 21 patients previously treated with Eculizumab.[37] Their results showed that regardless of prior use of Eculizumab, HSCT is still associated with 30% mortality, predominant causes being infections, and acute GVHD. The authors also highlighted the importance of close follow-up and surveillance for fungal infections in these patients post HSCT.[37,38]

Pediatric PNH accounts for 10% of total cases, and there are only a few published case reports and case series.[39-41] Children predominantly present with marrow failure rather than the classical variety.[39,40] Published studies on transplants in pediatric patients are summarized in Table 1.

Issues Related to Transplantation for Paroxysmal Nocturnal Hemoglobinuria

The only curative therapy available for PNH is HSCT, but it is associated with higher rejection rates as these patients have been heavily transfused previously and are alloimmunized. There is also high TRM.[23,34] Retrospective EBMT data, as stated previously has shown that transplant outcome is considerably worse for PNH patients with previous events of thrombosis.[42] The PNH clone can be successfully eradicated using appropriate conditioning regimens and through T-cell-mediated immunity of the graft against the abnormal PNH hematopoeitic stem cells.[36] Classical PNH can be treated efficiently using eculizumab, but it does not appear to treat the marrow failure component. In patients with marrow failure, life-threatening complications, disease transformation to MDS/AML, and transfusion-dependent hemolytic anemia HSCT is the only modality for cure.[43]

Type of donors

The choice of the donor is important for a successful transplant outcome. An identical twin donor is the ideal choice.[11] However, even with these donors, conditioning is still required to eradicate the PNH clone. Few case reports have shown that patients receiving no conditioning or only single-agent cyclophosphamide had a poor outcome and early relapse.[9,12] These data indicate that conditioning and immunosuppression in the recipient is needed even with syngenic donors. Andolina *et al*. showed that RIC is effective with syngeneic donors.[41]

Outcomes using matched unrelated donors (MUD) have been documented in previous studies.[28,29] In a CIBMTR study, 6 patients were transplanted with a MUD donor and only 1 was alive at the end of 5 years. The incidence of TRM is 15%–20% in unrelated and mismatched related donors (MRDs).[44] The use of haploidentical donors has increased over the past few years, and published literature suggests that haploidentical HSCT is feasible in PNH. Brodsky *et al*. published the first report of successful HLA-haploidentical HSCT in 3 PNH patients, out of which 2 achieved long-term survival.[30] Tian *et al*. reported the outcomes of 10 PNH patients who underwent haploidentical HSCT with 90% survival. The rate of acute GVHD was 40% and only one patient developed extensive chronic skin GVHD. The

Table 1: Studies with pediatric paroxysmal nocturnal hemoglobinuria patients undergone haematopoeitic stem cell transplantation

Reference	Number of patients	Type of donor	Conditioning	Outcome
Ware *et al.* (1991)[40]	1	NA	NA	1/1 alive
Graham *et al.* (1996)[15]	1	Syngenic	MAC	1/1 alive
Endo *et al.* (1996)[12]	1	Syngenic	None	1/1 alive
Flotho *et al.* (2002)[14]	2	MUD	MAC	1/1 alive
Curran *et al.* (2012)[39]	5	MSD (*n*=1), MUD/MMUD (*n*=2/2)	MAC	3/5 alive
Andolina *et al.* (2018)[41]	2	Syngenic (*n*=1), MUD (*n*=1)	RIC	2/2 alive

MUD=Matched unrelated donor; MSD=Matched sibling donor; MAC=Myeloablative conditioning; RIC=Reduced-intensity conditioning; NA=Not available

overall outcome and transplant-related complications were similar in the haploidentical HSCT and MRD transplants.[45] In another study, Xia *et al*. performed haploidentical HSCT for 17 patients using Bu-Cy-ATG conditioning regimen. All patients engrafted, and the median time for neutrophil and platelet engraftment was 12 days and 14 days, respectively. Seven patients developed acute GVHD, whereas 4 developed chronic GVHD.[46] In a recent single-center Chinese study, outcomes of 25 patients who underwent haploidentical transplants were reported. The authors documented a 3-year OS and GVHD-free failure-free survival of 86.5% ± 7.3% and 78.3% ± 8.6%, respectively.[43]

Type of conditioning

The PNH clone can be eradicated by both myeloablative and RIC regimens [Tables 2 and 3]. Myeloablative conditioning (MAC) regimens used in classical PNH consist of either a combination of busulfan, cyclophosphamide or fludarabine, or total body irradiation.[22,28] However, on the downside, MAC leads to increased nonrelapse mortality caused by the increased incidence of infection and sepsis. Hence, there has been a paradigm shift in the choice of the conditioning regimen. RIC regimens are advantageous for those patients preferring to maintain fertility and those with organ dysfunction who are poor candidates for MAC.[27] In PNH/AA overlap cases, cyclophosphamide/ATG is recommended for sibling transplants and fludarabine-based RIC for MUD HSCT.[48]

Previous studies have documented similar TRM and OS between recipients of RIC and MAC transplants.[34] In a recent study by Pantin *et al*., 15 of 17 PNH patients achieved long-term survival after Flu/Cy ± ATG-based MRD transplant.[36] Besides fludarabine, many transplanters have cited the favorable toxicity profile of treosulfan-based conditioning along with adequate myeloablation.[49] In addition, rates of acute and chronic GVHD are reduced.[29] In a Polish study, 21 patients of PNH or PNH/AA were transplanted using a treosulfan based conditioning.[50]

The feasibility of RIC regimens in PNH/AA overlap cases has been demonstrated. RIC regimens are capable of completely eradicating the PNH clone and thus achieving cure. In a recently published study by Lee *et al*., 33 cases of PNH underwent allogeneic HSCT. Twenty-one patients receiving RIC were followed up for 6 months posttransplant. The authors showed that RIC regimen caused PNH clone eradication by 2 months posttransplant and persistence of donor-type engraftment 6 months posttransplant.[51] In a Mexican study by Schcolnik-Cabrera *et al*., patients of hypoplastic PNH given a RIC regimen achieved an 8-year OS of 83.3%.[52] Future trials on the benefits of the RIC regimen for transplant in reducing TRM and achieving cure for PNH are warranted. Some authors have shown better outcomes in patients where eculizumab was administered as bridging therapy before HSCT.[47]

Stem cell source

Bone marrow grafts are preferred for benign hematological disorders. The same holds true for PNH. They are associated with reduced rates of GVHD.[36,47] T-cell depletion of peripheral stem cells using various *in vitro* and *in vivo* strategies have shown to reduce GVHD incidence.[53-55] Shasheleva *et al*., recently published their study using alpha/beta T lymphocyte depleted hematopoietic cells from matched unrelated donors in young adults with PNH. Eculizumab was used in the peritransplant period. They demonstrated normal hematopoiesis, full donor chimerism, and minimal late sequalae at a median follow-up period of 4 years posttransplant.[56] Umbilical cord transplants has also been performed by some researchers using RIC.[57]

Graft versus host disease

The incidence of GVHD is high post HSCT for PNH. Acute and chronic GVHD occurs in more than one-third of all patients.[58] Majority of the protocols use cyclosporine and methotrexate for GVHD prophylaxis.[23,31,32] In the study by CIBMTR group, the incidence of acute GVHD and chronic GvHD was 34% and 33% respectively.[23] Similarly, in the GITMO group, rates of acute and chronic GvHD were 42% and 50% respectively.[31] EBMT data showed the incidence of acute and chronic GVHD to be 40% and 29%, respectively. GVHD and infections were the main causes of death.[32] Liu *et al*. reported the cumulative incidences of acute GVHD grades II–IV and

Table 2: Myeloablative matched related donor Allo haematopoeitic stem cell transplantation transplants for paroxysmal nocturnal hemoglobinuria population

Reference	Number of patients	Conditioning	Outcome
Szer *et al*. (1984)[26]	4	Cy based	4/4 alive, median survival of 13 months
Bemba *et al*. (1999)[8]	16	Cy/TBI	5 years OS 58%
Saso *et al*. (1999)[23]	57	Cy based (*n*=56), none (*n*=1)	2 years OS 56%
Raiola *et al*. (2000)[23]	7	Bu/Cy	TRM 0%, median follow up 51 months
Lee *et al*. (2008)[9]	3	Bu-Flu-ATG	TRM 33%

TBI=Total body irradiation; OS=Overall survival; TRM=Treatment related mortality

Table 3: Nonmyeloablative Allo haematopoeitic stem cell transplantation for paroxysmal nocturnal hemoglobinuria population

References	Number of patients	Conditioning	Outcome
Hegenbart *et al*. (2003)[17]	7 (MUD=5, MRD=2)	Flu/TBI	4/7 alive at 13-38 months
Lee *et al*. (2003)[9]	2 (MMUD)	Bu/Flu/ATG, Cy/ATG	2/2 alive at 7 and 30 months
Takahashi *et al*. (2004)[27]	5 (MRD)	Flu/Cy/ATG	5/5 alive at 5-39 months
Brodsky *et al*. (2008)[30]	3 (Haplo)	Cy/Flu/TBI	2/3 alive
Tian *et al*. (2016)[45]	18 (10=Haplo, 4=MRD,4=MUD)	Cy/ATG with preconditioning	9/10 alive at 6-85 months
DeZern *et al*. (2018)[47]	8 (5=Haplo, 3=MRD)	Flu/Cy/ATG/TBI	5/5 alive at median follow up of 37 months

MUD=Matched unrelated donor; MRD=Matched related donor; TBI=Total body irradiation

chronic GVHD were 15.91% and 26.73% respectively.[43] As stated previously, incidence of GVHD is lower with bone marrow as the graft source. Majority of patients have been heavily transfused before transplant and hence develop HLA alloimmunization causing higher GVHD rates, more so with the use of peripheral blood stem cells.

Graft rejection and factors predicting survival

Clinical data with the use of MAC along with bone marrow grafts was associated with less rates of graft rejection.[59] With the use of RIC, risk of rejection would be more however, the risk of relapse will be lessened due to the 'graft versus PNH' effect, which has been discussed in more detail subsequently. A 100% donor chimerism is associated with complete disappearance of the PNH clone. Relapse occurs due to subsequent reappearance of the clone, as evidenced by increasing mixed chimerism in PNH patients.[51] Thus, a relapse of hemolytic PNH after allogeneic transplantation is possible and leads to secondary graft failure. Either a resistance of the PNH bone marrow or an altered immunological regulation can be putative causal factors.

In a study by DeZern *et al*., 10 patients with severe AA (SAA) had a PNH clone, which got eradicated after a RIC haploidentical HSCT.[60] Takahashi *et al*. studied the alloimmune status of PNH and normal cells in the RIC setting. The abnormal PNH granulocytes became undetectable in all patients by 4 months posttransplant. PNH cells remained sensitive to the T-cell mediated immunity, and this explains the immunological eradication of PNH cells post nonmyeloablative transplantation.[27] Similarly, Lee *et al*. showed that GPI-negative erythrocyte and granulocyte populations disappeared at median times of 1.3 and 2.2 months after transplantation, respectively.[51]

Socie *et al*., identified risk factors associated with a worse outcome. They found that four factors were associated with worse outcome, namely, presence of thrombosis (Relative risk [RR] 10.2), pancytopenia (RR 5.5), disease transformation (RR 19.1) and thrombocytopenia at diagnosis (RR 2.2).[61] Lee *et al*. studied the posttransplant outcomes of 33 PNH patients. Two patients underwent a second transplant in view of secondary graft failure. None of the surviving patients developed any secondary malignancies. The authors also demonstrated comparable outcomes of AlloSCT for PNH and SAA. The OS in this study was 87.9% at a median follow-up of 57 months. They also concluded that the OS is shorter for patients transplanted for classical PNH as compared to PNH/AA patients due to the PNH related comorbidities in the former.[51]

Patients undergoing MRD transplants have an OS of 50%–60%.[62] As per the EBMT data, a gap of more than 1 year from the time of diagnosis to transplantation was associated with poor survival outcomes.[32]

Graft versus paroxysmal nocturnal hemoglobinuria effect

The use of non-MAC with HLA-matched or haploidentical donors has resulted in the cure of PNH. This occurs due to the 'graft versus PNH' effect.[62] Pantin *et al*. conducted a pilot trial to evaluate the RIC HSCT in PNH. RIC enables the eradication of the abnormal hematopoeitic stem cells of the host without causing marrow toxicity, as observed with MAC. The concept behind graft versus PNH effect is that with a RIC (comprising of fludarabine/cyclophosphamide and

ATG), adequate lymphodepletion occurs and the graft with an appropriate dose of CD34 and donor T-cells leads to rapid engraftment and eradication of PNH positive recipient stem cells.[36]

Role of transplant in the era of eculizumab

Eculizumab is a C5 complement inhibitor and is efficacious in reducing intravascular hemolysis and risk of thrombosis in cases of classical PNH.[1,30] The two landmark trials, namely "TRIUMPH" and "SHEPHERD" trials have established the efficacy and safety of the drug.[63] On the downside, eculizumab is expensive, needs to be given lifelong and cannot eradicate the PNH clone nor provide a cure. It is not available in many low- and middle-income countries. Another drawback is the increased incidence of life-threatening *Neisseria* infections. HSCT remains the only therapy for those patients who do not achieve a good response to eculizumab. Eculizumab may not be effective in certa*in situ*ations like coexisting marrow failure or recurrent episodes of thrombosis.[44] The clinical picture of PNH is predominantly bone marrow failure in Asian patients as compared to thrombosis in the Western countries. Hence, HSCT plays an important role.[45]

Few groups have demonstrated the role of eculizumab before transplantation.[55] Recently, Cooper *et al*. showed that eculizumab given before transplant was associated with minimal GVHD.[64] Eculizumab use in the peri-transplant period also warrants study. Dezern *et al*. conducted a retrospective review of AA/PNH patients who got eculizumab before bone marrow transplant using non-MAC in combination with posttransplant cyclophosphamide for GVHD prophylaxis. Patients who received eculizumab before transplant had better outcomes in terms of transfusion independence and sustained engraftment.[47] Similarly, the French registry showed that patients previously treated with eculizumab had mortality reaching up to 30% regardless of the indications for HSCT in PNH, owing to infections and acute GVHD.[37]

A number of anti-complement therapies and biosimilars are in development. Ravulizumab (ALXN1210), a new complement C5 inhibitor, provides immediate, complete, and sustained C5 inhibition.[65] Other agents include Crovalimab (long-acting anti-C5 monoclonal antibody), LFG316, REGN3918, and coversin. Trials on inhibitors of proximal complement are also in the pipeline.[66] Although these options seem promising, the transplant will continue to be the modality of cure in the majority of patients in low- and middle-income countries where eculizumab and other novel therapies are still a distant dream.

In a study comparing eculizumab therapy and allotransplant therapy, it was shown that allotransplant is associated with lower costs and better survival, especially patients <35 years of age.[67] However, further prospective trials are needed to compare the two therapies. Although eculizumab is a highly effective therapy for PNH, there is still a subset of patients who cannot afford lifelong therapy, and nonresponsiveness in some patients also limits its use.[67]

The currently accepted indications for transplant in the eculizumab era include PNH with SAA/very severe AA, patients of MDS with a PNH clone and those who fail to respond to complement inhibitor therapy. In countries where eculizumab is not available, HSCT still remains the only curative option.

Special considerations during COVID 19 pandemic

Due to the current severe acute respiratory syndrome (SARS)-CoV-2 pandemic, it is essential to understand that patients of PNH are exposed to a significant risk of infection. In patients presenting with severe bone marrow failure, urgent HSCT is the treatment of choice. Hence, delaying the transplant is not a valid option. In such a scenario, screening of the donors and recipients by reverse transcription-polymerase chain reaction is required before starting the conditioning. Treatment protocols do not need any modification. In patients with moderate marrow failure, it is recommended to delay the transplant and manage conservatively. The use of thrombopoietin agonists like eltrombopag should be considered a bridge to definitive therapy like transplant or ATG administration. Patients with the classical PNH variety, eculizumab can be continued during the pandemic, as it is not known whether it predisposes the patient to SARS-CoV-2 infection. In patients who may develop the infection, severe breakthrough hemolysis can be seen and hence necessitate the modification of treatment schedule such as dose increments and shorter dosing intervals.[68]

Conclusions

Since HSCT in classical PNH is associated with high TRM, it should be offered to only those patients who are not responding to eculizumab. In developing countries where eculizumab is not available, patients can be considered for RIC HSCT with MRD/haploidentical donors with a possibility of "cure" due to the "Graft versus PNH" effect. The emergence of RIC to eradicate the PNH clone is an important milestone. RIC regimens containing treosulfan, fludarabine, and ATG are safe and effective without additional morbidity and mortality. Special considerations in view of the ongoing SARS-CoV-2 pandemic is recommended along with mandatory screening for donor and recipient before transplant. AA/PNH overlap patients are ideal candidates for HSCT and those cases of classical PNH

with life-threatening hemolysis or thrombosis where eculizumab is not easily available. With the advent of better RIC regimens, high-resolution HLA typing, advanced supportive care, transplants outcomes in PNH have improved over the years regardless of the type of donor, and in low- and middle-income countries like India where eculizumab is not available, transplant still remains the only cost-effective curative approach. Further investigational trials comparing HSCT versus medical therapy in PNH are reasonable.

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Conflicts of interest

There are no conflicts of interest.

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