Management of paroxysmal nocturnal hemoglobinuria

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Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired, chronic condition and is characterized by intravascular hemolysis, and is associated with bone marrow failure syndromes, particularly aplastic anemia and myelodysplasia. A single mutation of the *PIG-A* gene in bone marrow stem cells disrupts glycosylphosphatidylinositol (GPI) biosynthesis and results in a deficiency of all GPI-anchored proteins on the cell membrane and in the PNH phenotype. [1] *PIG-A* deficient cells are rapidly eliminated by autologous complement activation leading to many of the clinical manifestations of the disease[2,3].

Among the deficient proteins are the complement regulatory proteins CD55 and CD59. CD59 prevents terminal complement components from forming the hemolytic membrane pore, C5b-9 (the membrane attack complex). Absence of CD59 from PNH red cells results in intravascular hemolysis[3,4]. Hemolysis in patients with PNH can be monitored by levels of the enzyme lactate dehydrogenase (LDH). LDH is a standard biochemical measure of intravascular hemolysis and probably the most sensitive. The degree of hemolysis in PNH leads to consumption of endogenous nitric oxide (NO) by the free hemoglobin as well as through the release of erythrocyte arginase. Significant NO consumption has been demonstrated in patients with PNH[5] and is thought to contribute to smooth muscle dystonia, resulting in abdominal pain, dysphagia and erectile dysfunction, severe lethargy, renal impairment, pulmonary hypertension and thrombosis[3,6-9].

Increased mortality is recognized in patients with hemolytic PNH and is a consequence of the intravascular hemolysis and/ or thrombosis. Studies have demonstrated that approximately a third of patients on supportive therapies alone (folic acid, anticoagulation, blood transfusions) die within 5 years of diagnosis[10,11]; a statistic that compares to some hematological malignancies, indicating that PNH is not a benign disease. Ongoing hemolysis and/or insufficient hematopoiesis often result in transfusion dependence. However, some patients are able to compensate for the hemolysis by increasing erythropoiesis. They are, however, still at risk of the complications of the disease, and thereby the reduced survival, despite not requiring frequent transfusions. The most feared complication of PNH is venous thrombosis which occurs in approximately 50% of patients with hemolytic disease and is the cause of death in at least one-third[10,12,13]. Other organ dysfunction that can occur commonly as a consequence of the intravascular hemolysis and/ or thrombosis in PNH is renal impairment and pulmonary hypertension[14,15]. The symptoms may have a major impact on the patient's well-being. The acute exacerbations are usually unpredictable, and have a further adverse impact on quality of life.

Diagnosing PNH

Testing for PNH should be considered in patients with conditions listed in Table 1, including those with Direct Antiglobulin Test (DAT)-negative hemolytic anemia, unexplained cytopenia and unexplained thrombosis.

Flow cytometry is the principle investigative tool to diagnose PNH. Each laboratory tend to have a local preference for the reagents used and include monoclonal antibodies against CD55 and CD59 for red cells and CD14, CD16, CD24 and the reagent FLAER (fluorescein-labeled proaerolysin) for granulocytes and monocytes (Figure 1)[16]. Monitoring of the clone size by flow cytometry is also required in managing the disease and is most sensitive and specific when granulocytes are assessed as this lineage is unaffected by hemolysis and transfusion. Once detected, PNH clones, regardless of the initial size, should be closely monitored, usually 6-12 monthly[17].

Table 1. When to test a patient for PNH

Consider testing for PNH in patients with the following conditions:

- Patients with aplastic anemia and myelodysplasia
- DAT-negative (or complement only-positive DAT) hemolytic anemia
- Hemoglobinuria
- Recurrent abdominal pain or dysphagia with high LDH
- Thrombosis (venous or arterial) at an unusual site and/or in a young patient
- Unexplained cytopenia

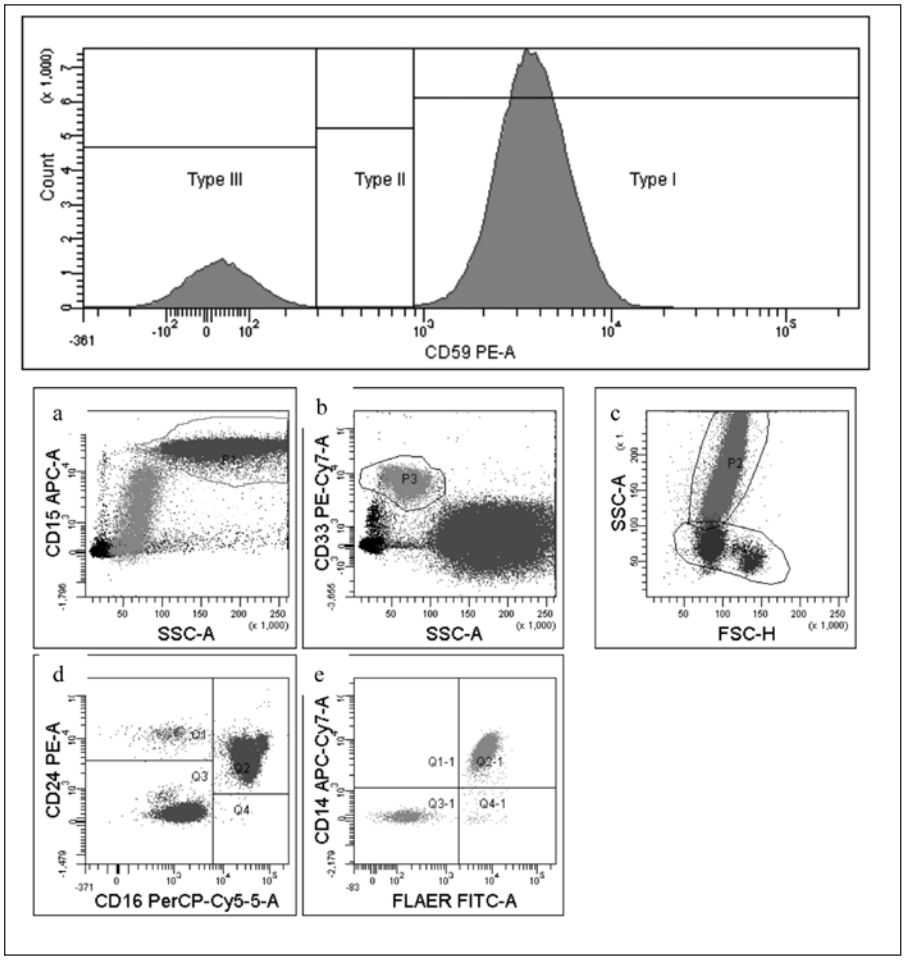

Figure 1. (Courtesy of MJ Cullen, Leeds Teaching Hospitals) **A) Histogram of PNH red cell analysis by flow cytometry** using anti-CD59 to demonstrate a large population of Type I (normal) red cells and a smaller population of Type III (completely deficient of GPI-anchored proteins) PNH red cells; **B) PNH granulocyte and monocyte analysis by multicolor flow cytometry using anti-CD14, CD15, CD16, CD24, CD33 and the FLAER (Fluorescent Aerolysin)** reagent. Granulocytes were identified using a combination of side scatter (SSC) and CD15 expression (Plot a, P1) and forward scatter (FSC) and SSC (Plot c, P2). Monocytes were identified using a combination of CD33 expression and SSC (Plot b, P3) and FSC and SSC (Plot c, P4). PNH granulocytes and monocytes are demonstrated in Q3 and Q3-1 of Plots d and e respectively.

Therapeutic strategies in PNH

Until recent years, the principle therapy for PNH has been supportive care with transfusions as required and the treatment of complications, such as thrombosis, when they occur (Table 2). It is conventional to give all patients with evidence of hemolysis folic acid supplementation as they will have increased red cell turnover. Many patients often also require supplementation with iron therapy, despite being transfusion dependent, due to the chronic hemosiderinuria and hemoglobinuria which may lead to iron deficiency. Corticosteroids have been used to reduce the hemolysis in PNH but high doses are required with its attendant side effects and therefore do not have a role in the long-term management of patients with hemolytic PNH.

The only curative strategy remains allogeneic stem cell transplantation but this carries a considerable risk of mortality.[18;19] In view of the fact that a proportion of patients will eventually experience a spontaneous remission of PNH ([10;20] and with the advent of effective novel therapies, stem cell transplantation should only be considered in selected cases, such as patients with a syngeneic donor or with predominant bone marrow failure.

Gene therapy is surprisingly not a logical therapeutic option in PNH patients as by targeting the *PIG-A* gene, the "corrected" PNH cells are probably likely to become to a target for the process that led to the bone marrow failure allowing the PNH clone to initially expand.

As complement mediated lysis or activation is responsible for the majority of the clinical manifestations in PNH, targeting complement is an attractive strategy in these patients. Replacing CD59, the deficient complement regulatory protein, has been attempted by several investigators as it is recognized that even low levels of CD59 on erythrocyte membranes are sufficient to protect the cells from lysis *in vitro*[21-24]. Alternatively, blocking the complement cascade is another strategy to prevent complement-mediated diseases or adverse effects. Perhaps surprisingly, deficiency of any of the complement molecules after C5 has little abnormal phenotype. The only apparent adverse effect is an increased risk of infection by the *Neisserial* species. However, terminal complement deficient individuals appear to have a lower mortality from such infections when compared to complement-replete individuals[25-27]. C5 would therefore seem an appropriate place to block the complement cascade and has been achieved with the drug, eculizumab (h5G1.1-mAb, Soliris, Alexion Pharmaceuticals)[28,29]. Eculizumab,

Table 2. Therapeutic strategies for patients with paroxysmal nocturnal hemoglobinuria

Supportive
• Red cell transfusions
• Folic acid and iron supplementation
• Anticoagulation
• Erythropoietin
• Corticosteroids
Disease modifying
• Allogeneic stem cell transplantation
• Complement blockade – eculizumab
• Replacing missing complement regulatory proteins
• Gene therapy
• Immunosuppression if bone marrow failure predominant

administered by intravenous infusion over 30 minutes every 2 weeks in the maintenance phase, is a recombinant humanized chimeric monoclonal antibody that specifically targets the complement protein C5 and prevents its cleavage. All patients who receive this drug should be vaccinated against *Neisseria meningitidis*.

Results from a 12-week open-label pilot study of eculizumab in patients with hemolytic PNH demonstrated a dramatic reduction in hemolysis, a marked decrease in the rates of paroxysms and blood transfusions and an improvement in quality of life. A 1-year follow-up study observed maintained reduction in LDH levels in both non-cytopenic and cytopenic patient populations[30]. The initial observations were extended to include clinical improvements in symptoms attributed specifically to hemolysis including hemoglobinuria, abdominal pain, dysphagia and erectile dysfunction. All completely resolved, or at least dramatically improved, during eculizumab treatment[31].

Following the pilot study, 2 Phase III trials were conducted. A randomized, placebo-controlled trial and an open-label, non-placebo controlled study designed to test eculizumab in a broader PNH population[32,33]. They demonstrate the high effectiveness of eculizumab in blocking chronic intravascular hemolysis and reducing or abolishing transfusion requirements. The improvements in hemolysis, fatigue, and transfusion requirements with eculizumab were independent of baseline levels of hemolysis and degree of thrombocytopenia.

Thrombosis rate, the leading cause of death, was evaluated in the 195 patients enrolled in the 3 clinical studies described above and its extensions[30,32-35] and demonstrated a significant reduction ($p < 0.001$)[36]. An important question, still to be addressed, is whether anticoagulation can safely be discontinued in patients with PNH who have had a previous thrombosis and are receiving eculizumab. This has been achieved successfully and reported in 3 patients although longer follow-up is required[37]. Data collected from the Global PNH Registry will hopefully also aid the answer and hematologists are strongly encouraged to enroll all PNH patients, regardless of clone size or therapy, into this Registry (www.pnhsource.com).

Additionally, eculizumab, through its ability to reduce NO consumption as well as prevent thrombosis, significantly reduces pulmonary hypertension as measured by BNP levels. Renal damage in PNH has been found to be common and associated with chronic hemolysis and subsequent hemosiderosis and/or microvascular thrombosis. Eculizumab treatment stabilizes or improves renal function in the vast majority of patients[5,36,38].

By preventing the intravascular hemolysis, subsequent nitric oxide consumption, thrombosis, renal damage and pulmonary hypertension, eculizumab would be expected to improve survival in patients with PNH. We were very encouraged by the findings of the recent study demonstrating that patients with hemolytic PNH on eculizumab have survival rates equivalent to a healthy, matched control population[11].

The most common adverse event with eculizumab therapy is headache, usually after the first or second infusion only. No clinically significant anti-eculizumab antibodies have developed in any treated PNH patients. The major concern, as described above, is that of increased risk of *Neisseria meningitidis.* Vaccination is required prior to therapy and our group also recommends prophylactic penicillin therapy as there is currently no vaccine cover for serotype B, the commonest strain of *Neisseria meningitidis* in the UK. Each treated patient also carries an alert card.

The significant benefits of eculizumab in a broad population of hemolytic PNH patients with varying preceding transfusion requirements have been clearly demonstrated. Although transfusions were significantly reduced and quality of life significantly improved, a proportion of patients may still require occasional transfusions and hemoglobin levels may not return to normal. Possible reasons include the underlying bone marrow failure or recognition of deposition of complement protein C3 on PNH red cells which may result in residual, low-level hemolysis as C5 blockade does not control for upstream effects of CD55 deficiency[39,40]. The latter phenomenon has recently been recognized with the use of eculizumab, as prior to blockage of terminal complement, these cells would most likely have been lysed intravascularly. Despite the revelation of this phenomenon, the benefit of blocking the intravascular hemolysis (and therefore reducing the cause of the complications and increased mortality) is maintained.

Table 3. **Summary points**

Summary

- PNH is a potentially disabling, lifethreatening disease
- Nitric oxide depletion contributes to smooth muscle dystonia (abdominal pain, dysphagia, erectile dysfunction), severe lethargy, renal impairment, hypertension and thrombosis

Diagnosis

- Consider testing for PNH in patients with AA or MDS, DAT-negative hemolytic anemia, hemoglobinuria and unexplained thrombosis
- Gold standard test for diagnosis is flow cytometry
- Clone size should be monitored regularly once found

Treatment

- All patients should receive folic acid supplementation
- Many patients require additional iron replacement
- Allogeneic stem cell transplantation remains the only curative strategy but indications have diminished in light of cases of spontaneous remissions of PNH and the advent of the effective therapy, eculizumab
- Eculizumab (Soliris) blocks the complement cascade at C5 preventing terminal complement activation. It effectively, safely and significantly prevents intravascular hemolysis thereby reducing (or abolishing) symptoms and transfusion requirements in patients with PNH
- Eculizumab significantly reduces thrombosis rate in patients with PNH
- Eculizumab improves renal function, reduces nitric oxide consumption and pulmonary hypertension in patients with PNH
- Eculizumab has safely been used in a small number of pregnant patients with PNH
- Eculizumab dramatically alters the natural course of PNH, reducing symptoms and disease complications as well as improving survival to the extent that it is equivalent to that of the general population
- All patients to receive Eculizumab should be vaccinated against *Neisseria meningitidis*
- All patients with a PNH clone, regardless of size or therapy, should be enrolled in the Global PNH Registry (www.pnhsource.com)

During pregnancy, PNH frequently worsens posing additional risk of morbidity and mortality to mother and fetus. Until recently, patients with PNH were strongly advised against pregnancy. Management was largely supportive and included prophylactic anticoagulation. Although, anticoagulation throughout pregnancy and the 6 week postpartum period is still advised, we now have some experience of the safe use of eculizumab in pregnancy with successful outcomes[41,42]. Although small numbers of patients, these findings are very encouraging.

Conclusion

There have been and continue to be exciting developments in the management of PNH. Eculizumab, the only FDA and EMEA approved drug for the treatment of PNH, has had a significant impact in improving the quality of life of patients with this potentially disabling and life-threatening disease as well as preventing the complications relating to the hemolysis. The drug effectively blocks terminal complement activation and has shown significant improvements in patients with PNH in terms of hemoglobin levels, transfusion requirements, thrombosis rate, quality of life, renal function, nitric oxide depletion, pulmonary pressure measurements and potentially pregnancy outcomes. By preventing the complications related to the intravascular hemolysis and dysregulated complement activation, exciting data demonstrates we are now in the era where it will be possible to normalise the survival for patients with PNH with eculizumab therapy.

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