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REVIEW ARTICLE

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How we treat paroxysmal nocturnal hemoglobinuria: A consensus statement of the Canadian PNH Network and review of the national registry

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Abstract

Paroxysmal nocturnal hemoglobinuria (PNH) is a rare hematologic disease characterized by intravascular hemolysis, thrombophilia, and marrow failure. Its phenotype is due to absent or reduced expression of GPI-linked complement regulators and subsequent sensitivity of hematopoietic cells to complement-mediated damage and lysis. Introduction of the terminal complement inhibitor eculizumab drastically improved outcomes in PNH patients; however, despite this improvement, there remain several challenges faced by PNH patients and physicians who care for them. One of the most important is increasing awareness of the heterogeneity with which patients can present, which can lead to significant delays in recognition. Data from the Canadian PNH Registry are presented to demonstrate the variety of presenting symptoms. In Canada, geography precludes consolidation of care to just a few centers, so management is distributed across academic hospitals, linked together as the Canadian PNH Network. The Network over the last several years has developed educational programs and clinical checklists and has worked to standardize access to diagnostics across the country. Herein, we address some of the common diagnostic and therapeutic challenges faced by PNH physicians and give our recommendations. Gaps in knowledge are also addressed, and where appropriate, consensus opinion is provided.

KEYWORDS

aplastic anemia and bone marrow failure, bone marrow transplantation, coagulation disorders, red cell disorders

1 | INTRODUCTION

Paroxysmal nocturnal hemoglobinuria (PNH) is a rare hematologic disease caused by somatic mutations in hematopoietic stem cells. These mutations in the phosphatidylinositol glycan-A (*PIGA*) gene cause a reduction or absence of glycosylphosphatidylinositol (GPI) expression on cell membranes and subsequently an absence of GPI-bound proteins.[1] Two GPI-dependent molecules that are absent are CD55 (decay accelerating factor; DAF) and CD59 (membrane inhibitor of reactive lysis; MIRL), both of which regulate complement activity. Erythrocytes lacking these proteins are exquisitely sensitive to complement-mediated intravascular hemolysis. Also, the unrestrained action of complement at the surface of hematopoietic cells, including platelets and leukocytes, initiates a complex chain of pathophysiological events that culminates in an increased risk of thromboembolic events (TE).[2] PNH is strongly associated with bone marrow failure (BMF) syndromes, such as aplastic anemia (AA) and myelodysplastic syndrome (MDS).[3]

Eculizumab is an anti-C5 monoclonal antibody.[4,5] Its use in PNH has significantly changed management and clinical outcomes.[6,7] Prior to the availability of eculizumab, PNH was managed mostly with supportive care (eg, transfusions, anticoagulation) and allogeneic stem cell transplant (ASCT). Eculizumab has been available in Canada since 2009. As of January 2018, our group collectively follows 67 patients on therapy and another 97 with small clones in the context of BMF. Approximately 52% of patients are female, and the median age of disease onset is 43 years. New complement inhibitors are currently at various stages of development,[8] and treatment options for PNH are likely to expand soon. Though there will certainly be molecule-specific considerations, the general concepts of PNH management should remain the same.

In Canada, as in other geographically large nations, care of patients with rare diseases can be challenging. Centralization of expertise is not possible to the same degree as in smaller countries. Clinicians may encounter several challenges, including recognition and diagnosis, access to testing, management of a disease with multisystem involvement, and drug reimbursement issues. The Canadian PNH Network (CPNHN) was established in 2012 to serve as a group of national experts to ensure current and consistent care for PNH patients. Herein, we provide our consensus approach to the recognition, diagnosis, and management of PNH. We will not describe in detail the underlying pathophysiology of PNH, as this has been addressed elsewhere in excellent and comprehensive reviews.[3,9] For this consensus statement, we did not employ a formal GRADE assessment for recommendations. Other than the randomized clinical trial TRIUMPH[5] and subsequent open-label SHEPHERD[4] assessing eculizumab, most evidence was considered moderate-to-low level. We therefore implemented a relative grading system, in which the phrasing "we recommend" denotes topics with more robust supporting evidence, whereas "we suggest" is used where advocacy is based on weaker evidence and consensus opinion.

2 | GENERAL MANAGEMENT OF PNH PATIENTS

2.1 | How is PNH diagnosed and what is involved in initial assessment?

The clinical presentation of PNH is heterogeneous. Patients can present with various symptoms or combinations thereof, with varying severity. Though the triad of hemolysis, TE, and BMF is characteristic, any number of symptoms may be present.[10] Fatigue, dysphagia, abdominal pain, dyspnea, dark urine, and erectile dysfunction may all occur, as can be seen in our Canadian cohort (Table 1). The non-specific nature of these symptoms can lead to delays in diagnosis and treatment. To facilitate more efficient case identification, we propose that the diagnosis of PNH should be considered in five clinical scenarios, abbreviated by the acronym CATCH: *c*ytopenias, *a*plastic anemia/myelodysplasia, *t*hrombosis, *C*oombs'-negative hemolysis, and *h*emoglobinuria (Table 2). Although this has not yet been formally evaluated as a screening tool, it has been in our experience a useful device for PNH case identification.

Initial assessment of a patient with suspected PNH includes investigations to confirm the presence of disease, its severity, and extent. This should entail a detailed history and physical examination with attention paid to specific signs and symptoms (Figure 1), including dyspnea, chest pain, and abdominal pain, as these are features independently associated with increased PNH-related mortality.[11] A history of TE, severe cytopenias, and renal insufficiency attributed to PNH also portend decreased survival without treatment.[11,12] Fatigue is another common symptom which may have been present yet unrecognized for years. This may be due to underlying anemia; however, fatigue out of proportion to the degree of anemia is often observed and likely reflects systemic nitric oxide depletion.[5,14]

Definitive testing for PNH is available. Several groups have published comprehensive guidelines,[15,16] though, as with most rare diseases, the greatest challenge at presentation is considering PNH in the differential diagnosis. Five main types of presentation (ie, CATCH) increase the likelihood of disease and should prompt consideration of testing.[15,19] To confirm the diagnosis, loss of GPI-linked structures from erythrocytes and leukocytes must be demonstrated using high-sensitivity flow cytometry. Guidelines have been published recently which review this in detail.[16,17,20] Briefly, two stainings are performed: one to show loss of CD59 expression on CD235a-gated erythrocytes and a second to show loss of GPI-linked structures on CD15-gated neutrophils and CD64-gated monocytes. GPI-linked structures such as CD24 (neutrophils), CD14 (monocytes), and CD157 and FLAER (both neutrophils and monocytes) are the most validated reagents used for leukocytes. These assays can detect PNH phenotypes with very high sensitivity (erythrocytes better than 0.01%, neutrophils better than 0.05%).[21] Since it is not uncommon to find a larger clone size in monocytes than neutrophils, the PNH clone reported should be the larger of the two

detected.[17] Type III (total loss of CD59), Type II (partial loss of CD59), and total CD59-deficient populations (ie, Types II + III) should be reported for erythrocytes, whereas only total GPI-deficient populations (neutrophils and monocytes) are reported for leukocytes (Figure 2). Though exceptions occur, patients with hemolytic PNH usually have Type III erythrocyte clones >5% and leukocyte clones >10%, whereas smaller GPI-negative populations are often found in the context of BMF.[10] In some cases with large clones, it is difficult to delineate Type II from normal Type I erythrocytes, a problem that can be exacerbated by prior transfusions. Addition of a CD71 conjugate to the erythrocyte assay allows simultaneous evaluation of reticulocytes alongside mature cells. It is relatively easy to delineate Type III from Type II, and Type II from Type I reticulocytes with this modification (DR Sutherland and A Illingworth, manuscript in preparation). In all cases, testing should be performed by modern, experienced laboratories following recommendations of the International Clinical Cytometry Society.[16,17,20,21]

Once PNH is identified, baseline laboratory assessment of a new patient is broad. Most will have at least some degree of hemolysis, often but not always associated with anemia. Younger PNH patients with a robust marrow response may not be anemic, but hemolysis can be recognized by the presence of reticulocytosis, elevated LDH, and undetectable haptoglobin. Previous blood counts, if available, may show relative anemia. Since intravascular hemolysis in PNH is not antibody-mediated, the direct antiglobulin test (DAT) in a treatment-naïve patient is expected to be negative. Severe or long-standing cases may be associated with iron deficiency, as brisk intravascular hemolysis leads to urinary iron loss. Hemoglobinuria is not universally reported (eg, 75.6% in the Canadian cohort), although it should be assessed by routine and microscopic urinalysis; in some, hemoglobinuria will have previously been misidentified as hematuria.[10,22,23] Hemolysis may not be readily identified in those with an associated BMF syndrome (eg, AA), in which reticulocytes will be pathologically suppressed, and the GPI-deficient erythrocyte mass may be small. In such

TABLE 1 Presenting disease characteristics of Canadian patients in the International PNH Registry[a]

Presenting symptom/sign[b]	All patients with PNH clone	Patients on eculizumab	Patients not on eculizumab
Elevated LDH ≥ 1.5 × ULN	43/107 (40.2%)	33/37 (89.2%)	10/70 (14.3%)
LDH ratio × ULN, mean (SD)	2.9 (3.6)	5.9 (4.5)	1.2 (1.2)
Granulocyte clone			
<10%	45/97 (46.4%)	1/32 (3.1%)	44/65 (67.7%)
10% to <50%	19/97 (19.6%)	3/32 (9.4%)	16/65 (24.6%)
≥50%	33/97 (34.0%)	28/32 (87.5%)	5/65 (7.7%)
Erythrocyte clone >10%	33/94 (35.1%)	25/31 (80.6%)	8/63 (12.7%)
Transfusion dependence	64/116 (55.2%)	21/24 (87.5%)	43/92 (46.7%)
BMF syndrome (%)			
AA	73/147 (49.7)	17/64 (26.6)	56/83 (67.5)
MDS	20/147 (13.6)	7/64 (10.9)	13/83 (15.7)
History of TE (%)			
Venous	16/144 (11.1)	12/65 (18.5)	4/79 (5.1)
Arterial	2/143 (1.4)	2/65 (3.1)	0/78 (0.0)
Abdominal pain	55/126 (43.7)	27/41 (65.9)	28/85 (32.9)
Dysphagia	22/126 (17.5)	11/41 (26.8)	11/85 (12.9)
Dyspnea	71/126 (56.3)	24/41 (58.5)	47/85 (55.3)
Pulmonary hypertension	3/140 (2.1)	2/66 (3.0)	1/74 (1.4)
Hemoglobinuria	49/125 (39.2)	31/41 (75.6)	18/84 (21.4)
Fatigue	111/126 (88.1)	40/41 (97.6)	71/85 (83.5)
ED in males	13/58 (22.4)	4/18 (22.2)	9/40 (22.5)

AA, aplastic anemia; BMF, bone marrow failure; ED, erectile dysfunction; HTN, hypertension; LDH, lactate dehydrogenase; MDS, myelodysplastic syndrome; SD, standard deviation; TE, thromboembolic event; ULN, upper limit of normal.

[a]Disease characteristics are presented at the time of starting eculizumab for "Patients on Eculizumab" and at the time of Registry enrolment for "Patients not on Eculizumab" and may not necessarily reflect initial presenting symptoms of disease depending on the time elapsed between symptoms, diagnosis, and registry participation.

[b]The total number (n) of cases and proportion (%) with available data are reported for each presenting symptom/sign. Alexion Pharmaceuticals provided scientific accuracy review of the International PNH Registry data presented (data downloaded January 2018).

patients, thrombocytopenia or neutropenia are noted, and the presence of additional cytopenias in a newly diagnosed patient should lead to investigation for BMF. In addition to laboratory testing, imaging may be indicated in the appropriate clinical context (eg, CT or ultrasound to identify TE, echocardiogram to assess pulmonary hypertension).

Recommendations

1. We suggest that a diagnosis of PNH be considered in patients presenting with otherwise-unexplained cytopenias, marrow failure, thrombosis, Coombs'-negative hemolysis, and/or hemoglobinuria.
2. We suggest that the initial evaluation of PNH patients include an assessment of disease extent and severity, with a detailed history, examination, and investigations as outlined in the Canadian PNH Network checklist (Figure 1).
3. We recommend high-sensitivity flow cytometry of peripheral blood as the accepted diagnostic standard, which should be performed with modern protocols in experienced laboratories.
4. We suggest that newly diagnosed PNH patients in Canada be referred to a Canadian PNH Network site for initial consultation to continue the work-up, to discuss treatment options, and to possibly enroll in research opportunities (eg, registries, trials).

2.2 | When and how should eculizumab be started?

Eculizumab is a humanized monoclonal antibody that binds to C5, the first molecule in the terminal complement cascade, whose activation culminates in formation of the membrane attack complex (MAC). Before its availability, median survival from diagnosis was only 10 years in a PNH cohort whose median age was 42.[24] In clinical trials, eculizumab has been shown to reduce hemolysis and transfusion dependence, improve quality of life (QoL), and extend overall survival (OS) in PNH patients.[4,5]

Eculizumab funding in Canada is approved in PNH patients who have a granulocyte and/or monocyte clone >10% with significant intravascular hemolysis (ie, LDH > 1.5 × the upper limit of normal [ULN]), accompanied by evidence of end-organ damage, including (1) significant anemia with or without transfusion dependence; (2) TE (venous or arterial); (3) pulmonary insufficiency or hypertension, unexplained by other pathology; (4) renal insufficiency unexplained by other pathology; or (5) recurrent abdominal pain or dysphagia necessitating hospitalization or opiate analgesia. Treatment may be considered for patients not meeting these guidelines, such as those with debilitating fatigue, or during pregnancy in a woman not already on eculizumab.[25] Patients without symptoms, regardless of clone size but especially those with small clones in the context of AA or MDS, do not need complement inhibition; however, they should be monitored regularly for the development of symptomatic PNH that may occur with GPI-deficient clonal expansion.[18,26] Occasionally, patients may develop hemolytic PNH while receiving immunosuppressive therapy (IST) for AA or hypoplastic MDS. Others may require IST for progressive marrow failure while established on eculizumab. Recent work suggests that overlap of these therapies, when required, does not undermine efficacy or increase infection risk.[27]

Eculizumab is an intravenous (IV) infusion. Standard induction in adults requires weekly dosing at 600 mg IV for 4 weeks. The dose is increased to 900 mg IV the fifth week and is subsequently given fortnightly.[4,5] Most adverse events are tolerable and diminish over time.[7] One persistent risk is infection from *Neisseria meningitidis* (0.42/100 patient-years);[7] the effectiveness of eculizumab to inhibit terminal complement creates a persistently increased susceptibility to meningococcal disease. The product monograph recommends vaccination with quadrivalent (vs serogroups A, C, W, and Y) and serogroup B vaccines at least 14 days before treatment unless the

TABLE 2 CATCH criteria as indications for PNH testing [15,18,22,26]

CATCH criterion	Indications for testing	Supporting information
Cytopenias	Patients for whom a bone marrow examination is considered for otherwise-unexplained cytopenia(s)	Additional features such as elevated LDH, DAT-negative hemolysis, history of unexplained TE, and hemoglobinuria
AA/MDS	All patients with a diagnosis or suspicion of AA. Testing should be done at diagnosis and monitored at least q6 months Low or intermediate-1 risk MDS, and especially if hypoplastic	Additional features such as elevated LDH, DAT-negative hemolysis, history of unexplained TE, and hemoglobinuria
Thrombosis	Unprovoked and/or unusual site TE (eg, splenic, hepatic, CNS), especially if recurrent and/or despite anticoagulation	Additional features such as elevated LDH, DAT-negative hemolysis, otherwise-unexplained cytopenias, especially including anemia
Coombs-negative hemolysis	Hemolysis or hemolytic anemia (ie, elevated LDH and indirect bilirubin, reduced haptoglobin, DAT/Coombs' test negative) without other clear cause	Test in all patients unless a clear alternate explanation exists. Supportive information may be helpful but is not necessary
Hemoglobinuria	Otherwise-unexplained hemoglobinuria or cases where "hematuria" has been identified without evidence of erythrocytes on microscopy	Test in all patients unless a clear alternate explanation exists. Supportive information may be helpful but is not necessary

AA, aplastic anemia; CNS, central nervous system; DAT, direct antiglobulin test (aka Coombs' test); LDH, lactate dehydrogenase; MDS, myelodysplastic syndromes; TE, thromboembolic event.

risk of delaying eculizumab outweighs infection risk.[28] Standard practice now is to start eculizumab as soon as possible based on clinical need and to use prophylactic antibiotics until 2 weeks after immunization (eg, penicillin VK).[3] In Canada, eculizumab is started before vaccine completion in 28.1% of cases. Repeat vaccination every 3-5 years is standard practice,[3] particularly as assays to evaluate immunity are not readily available. Many experts recommend long-term antibiotic prophylaxis post-vaccination for added coverage. This should be discussed with patients as it would theoretically provide additional protection; however, this protective effect has yet to be documented.

Vaccination against *N. meningitidis* serogroup B has been available in Canada since 2014. Use in those receiving eculizumab was quickly adopted, especially as serogroup B causes most meningococcal disease nationally.[29] The inflammatory response to vaccines may temporarily worsen markers of hemolysis (eg, LDH, haptoglobin) and even cause breakthrough disease (eg, anemia, hemoglobinuria);[30] however, the benefits of vaccination outweigh the short-term risks. PNH patients should be counseled on the signs and symptoms which may suggest breakthrough disease around the time of immunization. Supportive care with transfusions may be necessary. In more severe cases, an additional dose of eculizumab may be considered if available.

Recommendations

1. We recommend eculizumab be started in patients with a leukocyte PNH clone >10%, laboratory evidence of significant intravascular hemolysis, and at least one of: symptomatic anemia (regardless of transfusion dependence), thrombosis, renal insufficiency, pulmonary insufficiency or hypertension, or abdominal pain requiring admission or opioid analgesia.
2. We suggest eculizumab be considered in patients with a leukocyte PNH clone >10% and laboratory evidence of significant intravascular hemolysis who have disabling fatigue or who are pregnant.
3. We suggest that eculizumab and IST be used concurrently in situations where both are felt to be indicated.
4. We recommend that all patients who receive terminal complement inhibition be vaccinated against *N. meningitidis* using both quadrivalent and serogroup B vaccines.
5. We suggest that breakthrough hemolysis post-vaccination be managed with careful monitoring, though supportive care with transfusions may be necessary and, in more severe cases, an extra dose of eculizumab can be considered.
6. We recommend that patients started on terminal complement inhibition sooner than 2 weeks after meningococcal vaccinations be given prophylactic antibiotics to protect them while immunity is established.
7. We suggest meningococcal vaccinations every 3-5 years while patients remain on treatment.
8. We suggest that long-term anti-meningococcal antibiotic prophylaxis be considered if no contraindications exist.

2.3 | How do we monitor and evaluate PNH patients?

Most PNH patients respond well to eculizumab, with significant decreases in intravascular hemolysis and transfusion requirements, and improved QoL.[4,5,7] Response can be documented by reviewing the CBC, hemolytic markers, and other tests (Figure 3). Monitoring D-dimers following the start of eculizumab may be considered, particularly in those with increased TE risk or positive history; levels should fall rapidly with the start of eculizumab and remain suppressed,[31] though the utility of monitoring in PNH requires further research. Risk of pulmonary hypertension in PNH is increased. In the Canadian cohort, 3.0% of hemolytic PNH patients had a diagnosis of pulmonary hypertension (Table 1), though 58.5% described dyspnea, raising the possibility that some may have undiagnosed disease. Elevated N-terminal pro-brain natriuretic peptide (NT-proBNP) may identify patients at risk and appears to drop by 50% early after starting eculizumab,[32] but its role as a monitoring tool needs further study.

There is no universally agreed upon method to gauge therapeutic response. One group proposed definitions for complete response (CR), good partial response (GPR), and suboptimal response (SR; Table 3). Thirty patients were assessed over 863 patient-months of eculizumab therapy; four (13%) and 16 (53%) achieved CR and GPR, respectively.[33] It should be noted that repeat flow cytometry in a patient on eculizumab will likely show an increase in Type III erythrocytes; this does not signify worsening disease, but instead the anticipated effect of eculizumab blocking intravascular hemolysis, thereby extending erythrocyte lifespan.[17] The simplest way to evaluate efficacy is to assess hemolytic parameters immediately prior to the next eculizumab dose.[34] Patients should have near-normalization of LDH (ie, <1.5 × ULN). Direct measurement of trough drug concentrations has been suggested[7,35] but assays are not widely available. A more readily accessible approach has been proposed, in which complement function is tested using a 50%-hemolytic complement assay (CH50).[35] It remains to be demonstrated that measuring CH50 is of greater value than LDH measurement alone in routine clinical settings.

We monitor patients weekly when initiating eculizumab. After induction, patients can generally be seen monthly for the first few months until stable on therapy.[33] In 195 patients with PNH treated with eculizumab for 36 months, only 10.8% required a change in dosing.[7] Around 10%-15% of patients experience an increase in hemolytic markers toward the end of the 14-day cycle, and some may experience return of symptoms.[6] Despite eculizumab, approximately 30% may remain transfusion-dependent.[4] Physicians must be aware that eculizumab treats complement-mediated intravascular hemolysis only; patients may also have other causes of anemia (eg, BMF, iron deficiency, bleeding), and these need to be investigated and managed as would be done in any other patient.

The frequency with which patients with PNH clones should be monitored depends on several factors, including clone size and symptoms. Recently diagnosed patients starting eculizumab should be seen frequently to evaluate response. Those who are stable on treatment can be followed less often (eg, every

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Initial evaluation: Patient with newly diagnosed PNH

History and physical exam		Rationale for test/evaluation
Hemolysis	• When? • Frequency • Duration • Presence of hemoglobinuria • Management strategy • Precipitants	• Patterns and precipitants of hemolysis can help guide management
Fatigue		• In PNH, hemoglobin levels are not always correlated with fatigue; fatigue should be assessed independently of anemia
Thrombosis	1. When? 2. Where? 3. Complications 4. Management	• 40% of PNH patients experience thrombotic events (TEs) and TEs are the leading cause of death in PNH
1. Abdominal pain **2. Esophageal spasm** **3. Erectile dysfunction** (if applicable) **4. Pulmonary hypertension** **5. Renal insufficiency** **6. Iron status/overload** **7. History of fever/infections**	1. Yes/no 2. If yes, management	• Physical symptoms will help determine management strategy
Other comorbidities		• PNH commonly co-exists with aplastic anemia and MDS; other unrelated comorbidities may confound diagnosis and/or complicate management
Transfusion history	1. Yes/no; if yes, irradiated?	• Recent transfusion may confound RBC flow cytometry, as proportion of normal cells will be artificially high • Use of irradiated products is not standard practice for most patients in Canada but history may become relevant later for patients undergoing bone marrow transplantation
Medications	1. Soliris treatment history (if any) – see "For patients on Soliris" additions below 2. Other meds of interest: corticosteroids, anabolic steroids, vitamin supplementation (folate, vitamin D, calcium)	• Corticosteroids may have been previously used as empiric treatment for hemolytic anemia • Folate levels are often depleted in hemolysis due to increased erythropoiesis
Immune status	1. Allergies 2. General immunization history	• Penicillin allergy status and meningococcal vaccination history are particularly important if considering Soliris (see below)
Other	1. Female patients: Pregnancy history and future plans	• Pregnant women with PNH have an elevated risk of maternal and fetal morbidity and mortality; during pregnancy and post-partum there may be changes in transfusion, anticoagulation, and other medication requirements

MDS = myelodysplastic syndromes; RBC = red blood cells

FIGURE 1 PNH clinical checklist for initial diagnosis [Colour figure can be viewed at wileyonlinelibrary.com]

Laboratory evaluations

		Rationale for test/evaluation
Hemolysis	• Flow cytometry/FLAER • CBC, retic peripheral blood film • PT, PTT, D-dimer, fibrinogen • Iron: ferritin, TIBC • Direct antiglobulin test • Erythropoietin level	• Flow cytometry required to detect and quantify PNH clone • CBC to track anemia and other cytopenias • Elevated reticulocyte count indicates active hemolysis • PT, PTT, D-dimer, fibrinogen to assess thrombotic risk • Iron levels to monitor hemolysis; iron overload is rare but possible in chronically transfused PNH patients • DAT (Coombs test) should be negative to confirm that the hemolysis is not autoimmune in nature • EPO levels are naturally high in some PNH patients – often correlated with reticulocyte count
Organ function	1. Renal: GFR, urinalysis, microalbumin 2. Hepatic: LFT, LDH, bilirubin, haptoglobin 3. Cardiac: BNP (if available) 4. Bone marrow evaluation with cytogenetics	• Important to assess markers of organ damage at baseline; if stable/normal, ongoing monitoring does not have to be frequent • Bone marrow may be particularly relevant in patients with coexisting AA or MDS
Other	1. Viral serology: Hep A, B, C; HIV; CMV; HTLV1/2 2. Vitamin B12, folate (if available)	• Viral serology more relevant in transfused patients but should be done at baseline for all • Patients CMV-negative at baseline who require transfusions should receive CMV-negative blood products

Radiology

	Rationale for test/evaluation
1. Echocardiogram 2. Ultrasound abdomen with Doppler 3. Pulmonary CT if suspicion of pulmonary hypertension 4. Baseline bone density	• Echocardiogram and pulmonary CT to detect and assess pulmonary hypertension • Abdominal ultrasound to detect thrombi • Bone density particularly important in patients with prior steroid exposure

Additional evaluations: For patients on Soliris or other medications that increase meningococcal infection risk

History and physical exam

		Rationale for test/evaluation
Medications	1. Meningococcal prophylaxis (penicillin or other antibiotics)	• Prophylaxis recommended with Soliris treatment even if patient vaccinated
Immune status	1. Penicillin allergy 2. Meningococcal immunization history	• Penicillin is drug of choice for prophylaxis • Quadrivalent and serogroup B vaccines recommended before starting Soliris

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CBC = complete blood count; PT = prothrombin time; PTT = partial thromboplastin time; TIBC = total iron-binding capacity; DAT = direct antiglobulin test; EPO = erythropoietin; GFR = glomerular filtration rate; LFT = liver function tests; LDH = lactate dehydrogenase; BNP = B-type natriuretic peptide; AA = aplastic anemia; HIV = human immunodeficiency virus; CMV = cytomegalovirus; HTLV = human T-lymphotropic virus; CT = computed tomography

FIGURE 1 Continued

3-6 months). Reasons to intensify monitoring include an upcoming surgery or during pregnancy. Those with small PNH clones (ie, <10%) in the context of BMF should be followed based on recommendations for the underlying disease (eg, AA, MDS). In BMF patients it is important to monitor clone size with flow cytometry at least every 6 months;[18] clones can expand and cause classical PNH symptoms with attendant morbidity. Such patients should be counseled on signs and symptoms that may signify clonal expansion and the need for anticomplement therapy. Rarely, patients with hemolytic PNH may show spontaneous remission and no longer need eculizumab. In those situations, hematology follow-up is still recommended as there may be a risk of BMF or, less commonly, development of acute leukemia.[24,36]

By far, the most common cause of eculizumab failure is pharmacodynamic resistance, in which standard dosing is insufficient to maintain therapeutically effective levels. However, other resistance mechanisms have been documented. Development of human-anti-human or eculizumab-neutralizing antibodies as a cause of treatment failure has been evaluated,[7] but the incidence is low. Another uncommon cause of poor response to eculizumab is the presence of a polymorphism found mostly in Japanese patients.[37] Use of novel complement inhibitors may bypass this issue if they target a different C5 moiety or other complement molecule.[8]

Recommendations

1. We suggest that patients with newly diagnosed hemolytic PNH be monitored closely, particularly until eculizumab is started, to ensure no urgent intervention is necessary (eg, transfusions, anticoagulation).
2. We suggest that patients be evaluated for symptoms, transfusion requirements, and normalization of their CBC and LDH with each visit.
3. We suggest that PNH patients who remain transfusion-dependent be investigated for and treated for other potential causes contributing to the anemia.
4. We suggest that trough LDH and CH50 values be drawn immediately before a scheduled infusion to assess adequacy of the eculizumab dose (NB drug levels can be useful and may be available at some centers to help guide dosing).
5. We suggest more frequent follow-up in certain cases where increased disease activity may occur (eg, pregnancy).
6. We suggest that patients not on treatment but with a small PNH clone in the context of marrow failure syndromes be followed regularly with repeat flow cytometry, based on current guidelines.

2.4 | How do we manage breakthrough hemolysis?

Persistent or progressive anemia in an eculizumab-treated PNH patient could indicate breakthrough hemolysis, due to insufficient terminal complement suppression. Breakthrough may be an isolated and situational event, such as infection, surgery, or other trigger that can amplify complement activity. In this scenario, increasing eculizumab dose or frequency is rarely required; however, breakthrough should prompt investigation into the cause, and the patient should be monitored for recurrence. If breakthrough symptoms are more consistent, occurring near the end of each dosing cycle, increasing complement blockade should be strongly considered. Breakthrough hemolysis should be confirmed by checking hemolytic parameters and ensuring there is not another cause of anemia. Once breakthrough is confirmed, therapeutic options include increasing the dose of eculizumab (eg, 1200 mg every 14 days) or shortening the time between infusions (eg, every 12 days). This should lead to appropriate complement inhibition in 98% of patients.[7] Our practice is to first increase the dose, leaving the frequency unchanged; practically, it is easier for patients and infusion centers to keep the same day of the week for treatments.

Persistent evidence of hemolysis in an eculizumab-treated patient may suggest extravascular hemolysis, caused by increased C3 deposition on erythrocytes. Eculizumab blocks MAC formation; however, unrestrained activation of proximal complement pathways, unaffected by eculizumab, causes C3 split products to accumulate and opsonize erythrocytes, marking them for extravascular destruction.[38] In this situation, the DAT will be positive for C3d. This is not seen in PNH patients not receiving eculizumab.[39,40] In some, extravascular escape can lead to symptomatic anemia, but it is not considered breakthrough disease; instead, it is an iatrogenic effect of terminal complement blockade, and its management is different. Strategies include use of corticosteroids and splenectomy, though neither is very effective, and each has potential complications.[3] This may be avoided in the future with the use of novel C3 inhibitors.[8]

Recommendations

1. We suggest that, if breakthrough disease occurs, the cause and pattern of occurrence should be monitored. For isolated instances, no change in eculizumab dosing is necessary.
2. We suggest that regular breakthrough hemolysis be managed by either increasing the dose of eculizumab or reducing the time between infusions.
3. We suggest that extravascular hemolysis, which can occur in patients receiving eculizumab, be identified by a newly positive DAT (C3d+). Treatment with corticosteroids or splenectomy can be considered but the risks and benefits of either approach must be weighed carefully.

3 | MANAGEMENT OF THROMBOSIS IN PNH

3.1 | What is the impact of eculizumab on thrombotic outcomes?

Thromboembolism remains one of the major causes of death in PNH patients.[2] Although the clinical trials did not include TE as a primary

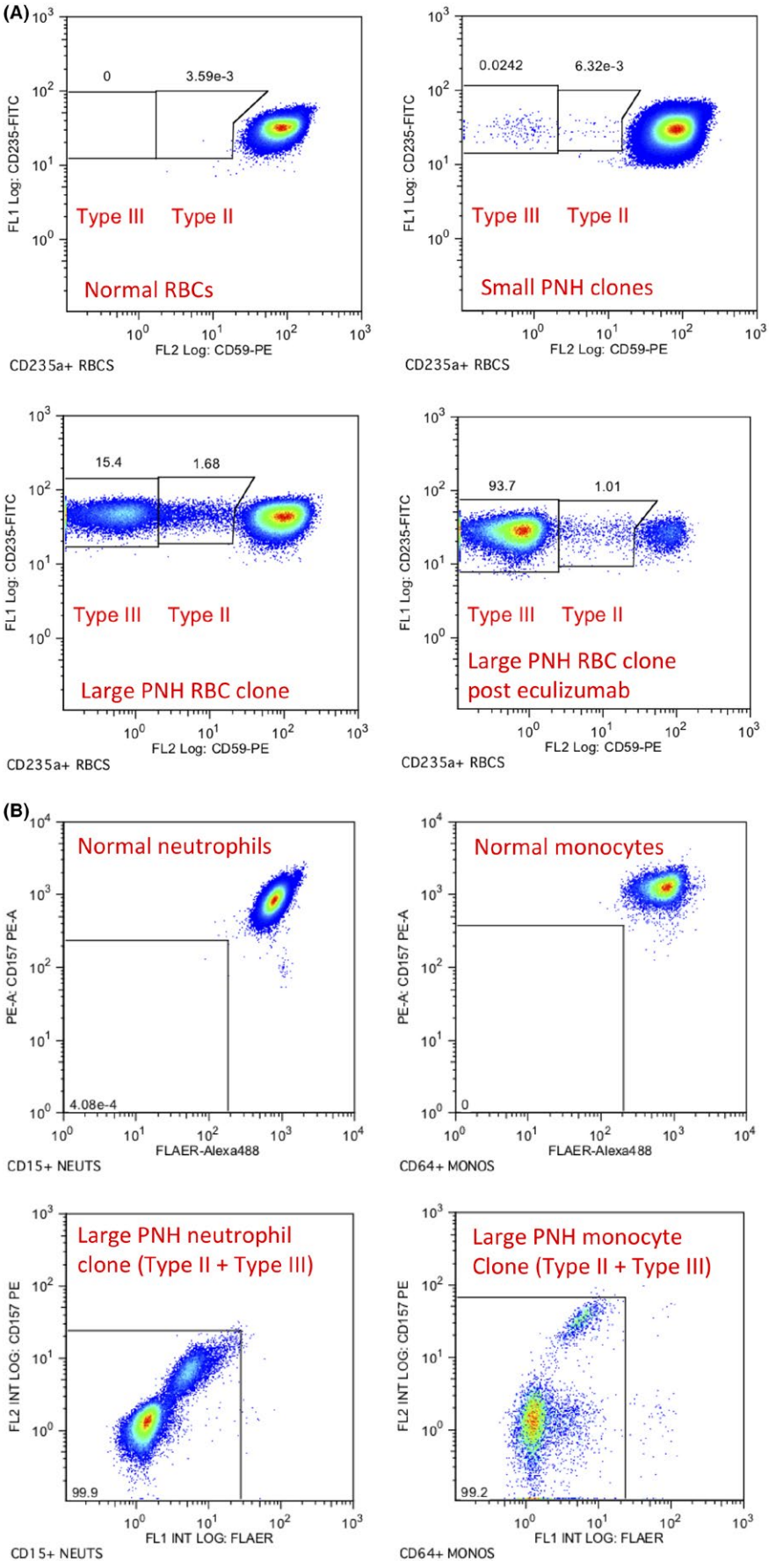
(A)
0
3.59e-3
Type III
Type II
Normal RBCs
FL1 Log: CD235-FITC
FL2 Log: CD59-PE
CD235a+ RBCS
0.0242
6.32e-3
Type III
Type II
Small PNH clones
FL1 Log: CD235-FITC
FL2 Log: CD59-PE
CD235a+ RBCS
15.4
1.68
Type III
Type II
Large PNH RBC clone
FL1 Log: CD235-FITC
FL2 Log: CD59-PE
CD235a+ RBCS
93.7
1.01
Type III
Type II
Large PNH RBC clone
post eculizumab
FL1 Log: CD235-FITC
FL2 Log: CD59-PE
CD235a+ RBCS
(B)
Normal neutrophils
4.08e-4
PE-A: CD157 PE-A
FLAER-Alexa488
CD15+ NEUTS
Normal monocytes
0
PE-A: CD157 PE-A
FLAER-Alexa488
CD64+ MONOS
Large PNH neutrophil
clone (Type II + Type III)
99.9
FL2 INT LOG: CD157 PE
FL1 INT LOG: FLAER
CD15+ NEUTS
Large PNH monocyte
Clone (Type II + Type III)
99.2
FL2 INT LOG: CD157 PE
FL1 INT LOG: FLAER
CD64+ MONOS

FIGURE 2 Flow cytometric analysis of normal and PNH erythrocyte and leukocyte samples. A, Erythrocytes. Normal sample (top left), patient sample with very small PNH erythrocyte clones (top right), patient sample with large PNH erythrocyte clones (bottom left), and patient sample with large PNH erythrocyte clones after 1 y of treatment with eculizumab (bottom right), stained with CD235a-FITC (glycophorin-a) and CD59-PE. Samples processed/acquired and analyzed as detailed in the references.[17,19,20] B, Leukocytes. Normal sample (top row) stained with 5-color leukocyte assay comprising FLAER, CD157-PE, CD15PerCPCy5.5, CD64-APC, and CD45 eFluor450.[21] Normal CD15-gated neutrophils (top left) stain with CD157 and FLAER. Normal CD64-gated monocytes (top right) stain with CD157 and FLAER. PNH patient sample (bottom row) stained with same 5-color reagent set. CD15-gated neutrophils (bottom left) show almost 100% PNH neutrophils comprising both Type III and Type II phenotypes. CD64-gated monocytes (bottom right) also show almost 100% PNH monocytes comprising both Type III and Type II phenotypes. NB: These figures are intended to illustrate the different presentations one may see; ordering physicians do not themselves have to analyze these plots, nor will they usually be included in a final report

outcome,[4,5] observational data have consistently demonstrated a protective effect of eculizumab.[6,7,41] Hillmen et al combined the initial trial cohorts and assessed TE relative risk reduction (RRR) in a prespecified analysis. There was an 85% RRR after starting eculizumab in the overall cohort, without adjustment for time spent on therapy. In the subpopulation receiving anticoagulants, the RRR was 94% (11.54 vs 0.72/100 patient-years). Similar findings were noted in all subpopulations and were statistically significant.[41] Protective effects of eculizumab have also been documented by others.[6,7] Despite this, Loschi et al have shown that a history of TE remains a significant factor reducing overall survival in PNH (HR 4.48, 95% CI 2.46-8.16), even in those receiving eculizumab.[42]

3.2 | What is the role for anticoagulation in PNH?

Before pharmacologic complement inhibition, anticoagulation was standard management to prevent TE in PNH. Treatment included therapeutic-dose heparin with transition to warfarin. This was a common practice for those with a history of TE, but also as primary prophylaxis. A granulocyte clone ≥50% was often used as a threshold above which to start warfarin, as work in the pre-eculizumab era demonstrated significantly greater TE risk with larger clones.[43] Today, the role of anticoagulation in PNH depends on TE history and availability of eculizumab. In patients with elevated LDH but who do not satisfy eligibility criteria for eculizumab, one may consider primary prophylaxis.[43] Patients who start eculizumab without a history of TE do not need anticoagulation and can discontinue primary prophylaxis.[6] For PNH patients with a history of TE, anticoagulation should be maintained long-term unless there is reason to stop (eg, bleeding, severe thrombocytopenia).[2]

Thromboembolism should be treated as per standard guidelines,[44] with the only difference being that there is minimal evidence for direct oral anticoagulants in PNH [45]; however, some physicians will use these instead of warfarin for their ease of administration and lower bleeding risk.[46] As is the case with any TE, one must carefully consider the risks and benefits of treatment. In this regard, PNH patients may be particularly challenging if there is concomitant thrombocytopenia from BMF.[2] In addition to anticoagulation, patients with new TE should be started urgently on eculizumab to reduce the major driver of their prothrombotic state (ie, dysregulated complement activity).

Recommendations

1. We recommend that history of TE in the context of PNH is an indication to start complement inhibition, as this significantly reduces the risk of progression and prevents future TE as well.
2. We suggest that patients without a history of TE do not require primary prophylaxis with anticoagulation if they start eculizumab.
3. We suggest that patients with a history of TE continue anticoagulation, as well as eculizumab, unless there exists a clear reason to stop (eg, bleeding, severe thrombocytopenia).
4. We recommend that development of new TE in a PNH patient should prompt immediate initiation of therapeutic anticoagulation, as well as eculizumab if not already prescribed.

4 | MANAGEMENT OF SPECIAL POPULATIONS

4.1 | How should PNH be managed during pregnancy?

Historically, PNH patients wishing to become pregnant were often advised against it.[47,48] In the pre-eculizumab era, maternal mortality was approximately 8%-21%, with most deaths from TE. Fetal outcomes were also worse compared to healthy comparisons; only half of pregnancies progressed to term, and fetal mortality was 4%-9%.[48,49] Several studies have documented increased complement activity even in normal pregnancies and preeclampsia.[52] In PNH patients, in whom baseline complement regulation is dysfunctional, most aspects of the disease (eg, hemolysis, transfusion dependence) are exacerbated by pregnancy.[53,54] In addition, cytopenias in the context of AA may worsen with pregnancy, so increased monitoring is required for these patients as well.[18]

Prospective data for the treatment of pregnant PNH patients are not readily available, as pregnancy was an exclusion for the eculizumab trials.[4,5] Prior to 2015, evidence was based on case reports[55,56] and a small series.[57] In 2015, Kelly et al published the experience of the global PNH community, reporting outcomes of 75 pregnancies in 61 women.[25] No maternal deaths occurred, and fetal mortality was 4%. Increased transfusion dependence was seen, compared to baseline. Just over half of mothers who

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Follow-up of PNH patients after initial workup – routine visit (every 3-4 months) for all patients, regardless of Soliris treatment

Frequency determined by treatment, disease severity and local support

History and physical exam

Monitor the "6 P's":
1. Pep: rating fatigue
2. Paroxysms: have there been any episodes of increased hemolysis or of hemoglobinuria
3. Pallor: anemia, transfusions
4. Pulmonary: dyspnea
5. Pain: esophageal spasm, chest/abdominal pain, need for narcotic analgesia
6. Penis: erectile dysfunction (if applicable)

Rationale for test/evaluation
- Physical symptoms will help determine management strategy

Hematology

1. FLAER/RBC flow
2. CBC
3. Reticulocytes
4. LDH
5. DAT
6. D-dimer
7. Serum ferritin

Rationale for test/evaluation
- Flow cytometry required to monitor any expansion of the PNH clone
- CBC to track anemia and other cytopenias
- Reticulocytes and LDH to detect active hemolysis
- DAT to confirm that hemolysis is not autoimmune
- D-dimer to assess thrombotic risk
- Serum ferritin to assess potential iron overload or deficiency – if normal, it can be tested less frequently (e.g., every 6 months)

Renal

1. Electrolytes
2. Creatinine, estimated CrCl
3. Microalbumin
4. Urinalysis (routine & microscopic)

Rationale for test/evaluation
- Important to compare markers of renal function across visits to assess any deterioration

Other

1. BNP
2. Previous Soliris treatment history (if any) and outcomes

Rationale for test/evaluation
- If BNP stable/normal, ongoing monitoring can be less frequent (e.g., annually)

RBC = red blood cell; CBC = complete blood count; LDH = lactate dehydrogenase; DAT = direct antiglobulin test; CrCl = creatinine clearance; BNP = B-type natriuretic peptide

FIGURE 3 PNH clinical checklist for follow-up visits [Colour figure can be viewed at wileyonlinelibrary.com]

Follow-up – additional evaluations at routine visit for patients on Soliris

History and physical exam

	Rationale for test/evaluation
An additional "2 P's": 1. Pyrexia: history of fever/infections 2. Prophylaxis: history of penicillin or other antibiotics for meningococcal prophylaxis, requirement for new/renewed prescription	• Patients on Soliris should report any infections/fevers they experience • Prophylaxis recommended with Soliris treatment even if patient vaccinated

Additional evaluations – to be done at least once in patients on Soliris

Other

	Rationale for test/evaluation
1. Anti-meningococcal titres (once available)	• Antimeningogoccal titres coming soon to Canada; will help assess whether prior vaccination provided effective protection

Additional evaluations – annual visit (all patients, whether on Soliris or not)

Other

	Rationale for test/evaluation
1. 2-D echocardiogram	• Monitor cardiac function and assess for pulmonary hypertension

Additional evaluations – patients with small/asymptomatic clone

Hematology

	Rationale for test/evaluation
1. Evaluate clone size every 6-12 months	• Monitor any changes in clone size and how they correlate to development of symptoms

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FIGURE 3 Continued

TABLE 3 Response assessment criteria for patients with hemolytic PNH[33]

Response category	Definition
Complete response	Transfusion independence Normal hemoglobin for age and sex Absence of symptoms LDH < 1.5 × ULN
Good partial response	Decreased transfusions from pretreatment status LDH <1.5 ULN No TE since start of therapy Hemoglobin remains low for age and sex
Suboptimal response	Unchanged transfusion needs Persistent/new symptoms since start of therapy

LDH, lactate dehydrogenase; TE, thromboembolic event; ULN, upper limit of normal.

progressed beyond the first trimester required increased eculizumab. Anticoagulation was used in 89%; no TEs occurred during pregnancy, two occurred immediately postpartum, and two occurred in cases of eculizumab discontinuation. Low eculizumab concentrations, at levels not expected to inhibit complement, were detected in one third of available cord blood samples, and no eculizumab was detectable in breast milk.

PNH patients planning to get pregnant should be counseled on the risks of continuing with and without eculizumab.[28] Though observational, most data suggest that eculizumab is reasonably safe in pregnancy and results in control of PNH, which can itself have untoward effects on mother and child. Patients should be monitored closely, especially past the first trimester. Elevated LDH, hemoglobinuria, increased transfusion dependence, TE, and other symptoms should prompt consideration of increasing the eculizumab dose or frequency. Thromboprophylaxis should be prescribed barring contraindication. Most mothers in the international series received LMWH, about half at a prophylactic dose.[25] No evidence-based recommendations can be made about anticoagulant dose or duration, but most experts adhere to standard guidelines, continuing therapy to 6 weeks postpartum unless there is a reason to extend.[58] Because of the increased morbidity and mortality with PNH alone, and with antepartum anticoagulation, high-risk obstetrics should be included early in the care plan.

Recommendations

1. We suggest that eculizumab be continued in pregnant PNH patients after reviewing the risks and benefits, as PNH symptoms commonly increase in pregnancy, and current data do not support a clear risk to the newborn.
2. We suggest close follow-up of pregnant PNH patients, especially once past the first trimester, as over 50% will require an increased dose and/or frequency of eculizumab until delivery.
3. We suggest prophylactic or therapeutic anticoagulation with LMWH be started in pregnant PNH patients, and continued until at least 6 weeks postpartum, unless there is a clear contraindication.
4. We suggest high-risk obstetrics be involved early for any PNH patient planning a pregnancy.

4.2 | What is the role of allogeneic stem cell transplantation in PNH?

The only curative therapy for PNH is ASCT, as the clone is eradicated by the conditioning regimen and engraftment of donor cells. A T-cell-mediated immune effect against PNH stem cells has also been demonstrated.[59] PNH was one of the earliest conditions treated by ASCT[60] and, despite its rarity, has been the subject of numerous case series and reports, as transplantation methods, donor selection, and conditioning have evolved. More recently, the therapeutic approach to PNH has changed, as eculizumab is associated with significantly less toxicity and is widely available. This requires a reassessment of indications, timing, and patient selection for ASCT.[61]

Historically, reasons for considering ASCT in PNH were severe hemolysis, life-threatening TE, and BMF. The first case series reported four patients, each with PNH in the aplastic phase[62,63] with long-term engraftment and no recurrence. This early success led to recognition of the curative potential for PNH, and ASCT was widely adopted. In 1999, the Hôpital St Louis in Paris described 16 patients who received related-donor ASCT; most were transplanted for BMF, three had severe hemolysis, and none had prior TE. A variety of cyclophosphamide and total body irradiation (TBI)-based preparative regimens were used. The 5-year OS was 58%, with acute GVHD in 50% and chronic GVHD in 21%.[64] A report on 57 patients in the International Bone Marrow Transplant Registry confirmed these results, with 56% 2-year OS, and 34% developing acute and chronic GVHD.[65] Most donors were matched siblings. In the unrelated group (n = 7), only one patient was alive at 5 years. Two recipients of syngeneic transplants were alive and well. The outcomes of this group who received pretransplant conditioning contrasts with earlier reports where simple marrow infusion without conditioning led to non-engraftment or recurrence in most cases.[66,67] Experience with syngeneic donors indicates that some degree of immunosuppression of the recipient is important, even when there is no HLA barrier to engraftment.

Motivation to decrease conditioning toxicity and treatment-related mortality (TRM) led to reduced-intensity conditioning (RIC) transplantation,[70] but results are mixed. Favorable results have been noted in several series: 4 of 7 with long-term disease-free survival using fludarabine and TBI in one series,[71] and 15 of 17 after cyclophosphamide,

fludarabine, and ATG in another.[72] These results were not confirmed by the Italian co-operative group who described 26 patients,[73] of whom 16 received RIC transplantation: TRM was higher in the RIC group (63%) compared to 26% receiving myeloablative regimens. Of note, this study may not be representative, as it spanned many years (1988-2006) during which patient selection, matching, GVHD prophylaxis, and supportive care evolved significantly. In recent years, there has been renewed interest in the use of haploidentical donors and post-transplant cyclophosphamide. Tian et al reported that 9 of 10 patients undergoing myeloablative haploidentical transplantation for PNH were alive at a median follow-up of 20 months.[74]

The largest study to date of ASCT in PNH was published by de Latour et al in 2012.[75] Outcomes of 211 patients between 1978 and 2007 were reported. It is the only study to directly address outcomes according to ASCT indication, and to compare these to treatment with standard therapy in matched controls. It must be noted that the time period analyzed pre-dated eculizumab. As such, improved survival in non-transplanted patients would be expected with complement inhibition.[6] The median age was 30 years, and transplants were done for BMF in 62%, hemolysis in 70%, and TE in 25%. HLA-identical siblings were available in 65%, and 64% used marrow as the graft source. Conditioning was cyclophosphamide-based in 70% and fludarabine-based in 30%. At 5 years, 40% had grade 2-4 acute GVHD and 29% had chronic GVHD. Overall survival was 68%, but varied by indication; survival was best for hemolysis (86%), followed by BMF (69%), and worst for patients transplanted for TE (54%). These results call into question the utility and safety of transplanting patients with PNH and TE. Conversely, outcomes in those with BMF are consistent with results in AA and suggest there is an important role for ASCT in this setting.

Whether patients should receive pretransplant immunosuppression is difficult to discern from registry studies. This should be decided based on algorithms for treatment of patients with AA without a PNH clone. There are limited data to support use of eculizumab around the time of ASCT to manage transplant complications. Some support its use up until conditioning, particularly in the context of thrombotic risk;[76] however, it is not clear that it changes overall outcomes.[77] Outcomes reported in the literature span different eras, variable patient and donor selection, and a diversity of conditioning regimens. Survival and QoL have improved over time but, in most situations, would likely be inferior to eculizumab, and a proportion of ASCT survivors will suffer from GVHD and other complications.[77] True eculizumab failure (eg, persistent intravascular hemolysis, recurrent TE) is uncommon and may be associated with polymorphisms,[37] the consequences of which may be bypassed using novel agents.[78] Since eculizumab became available in Canada in 2009, the authors have not resorted to ASCT for any patient with hemolytic PNH as their primary disease.

Recommendations

1. We suggest that ASCT not be considered standard of care for patients with hemolytic PNH, nor in patients with thromboembolism.
2. We suggest that ASCT be considered in patients with severe aplastic anemia and presence of a PNH clone, according to the same algorithms used for patients with severe aplastic anemia alone.
3. We suggest that ASCT be considered in PNH patients with evidence of clonal evolution (eg, MDS, leukemia).

5 | THE CANADIAN PNH NETWORK

The CPNHN is composed of hematologists from 12 academic centers across the country with expertise in the management of PNH. The impetus for creation of the CPNHN came from recognition of the rarity of PNH, and that one or a few centers in Canada could not see most patients in the country, as may be the case in smaller nations. Representation from different provinces allows sharing of experiences, including availability of diagnostics and treatments. Members of the CPNHN meet in person at least once annually and via webinar. We use online meetings to discuss challenging cases, present new evidence, and review projects.

The CPNHN website (www.PNHnetwork.ca) provides information on topics related to PNH diagnosis, testing, and management. One feature, PNH Connect, allows physicians to schedule case conferences with Network members. We also provide clinical assessment templates that may be used at initial and follow-up visits. Finally, the website provides information on how to enroll patients in the International PNH Registry.

6 | DISCUSSION

Though recognition of PNH has increased since eculizumab became available, it remains a rare disease with heterogeneous presentations. Diagnosis requires a heightened index of suspicion, and patients may make first contact with various medical specialties depending on the presenting symptoms. Once a diagnosis is made, those with hemolytic PNH and/or patients with PNH and TE should be considered for eculizumab or clinical trials of other complement inhibitors. Those with small, clinically asymptomatic clones require regular follow-up for monitoring clonal expansion and evolution, as well as for management of their underlying condition. Beyond initiation of anticomplement therapy, PNH patients require regular assessments to review disease control, vaccination status, possible transfusion support, and planning around scenarios that will potentially increase complement activity. Eculizumab is still the only Health Canada-approved therapy for PNH, but many others are under investigation at various stages. Efforts to standardize diagnostic and therapeutic approaches, promote research activity, and education of our colleagues are important goals of the CPNHN. Its members are open to discuss patient cases, see them in initial consultation, and either assume or share care with referring colleagues.

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