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REVIEW ARTICLE

Consensus recommendations for optimising the diagnosis and treatment of paroxysmal nocturnal haemoglobinuria in Singapore

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ABSTRACT

Introduction: Paroxysmal nocturnal haemoglobinuria (PNH) is a rare haematologic disease characterised by intravascular haemolysis, thrombophilia and bone marrow failure. There is a lack of established clinical guidance on the screening, diagnosis and management of PNH in Singapore. A relatively low level of awareness among healthcare professionals regarding PNH manifestations further contributes to diagnostic delays. Additionally, limited access to complement inhibitors, like eculizumab, may delay treatment and impact patient outcomes.

Method: Nine haematologists from different institutions in Singapore convened to formulate evidence-based consensus recommendations for optimising the diagnosis and management of patients with PNH and improving access to novel treatments. The experts reviewed the existing literature and international guidelines published from January 2010 to July 2023, focusing on 7 clinical questions spanning PNH screening, diagnostic criteria, investigations, treatment and monitoring of subclinical and classic disease, PNH with underlying bone marrow disorders, and PNH in pregnancy. A total of 181 papers were reviewed to formulate the statements. All experts voted on the statements via 2 rounds of Delphi and convened for an expert panel discussion to finetune the recommendations.

Results: Sixteen statements have been formulated for optimising the screening, diagnosis and management of PNH. Upon confirmation of PNH diagnosis, individuals with active haemolysis and/or thrombosis should be considered for anti-complement therapy, with eculizumab being the only approved drug in Singapore.

Conclusion: The current recommendations aim to guide the clinicians in optimising the screening, diagnosis and management of PNH in Singapore.

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Keywords: aplastic anaemia, bone marrow failure, complement inhibitors, intravascular haemolysis, practice guidelines

CLINICAL IMPACT

What is New

- Diagnosis of paroxysmal nocturnal haemoglobinuria (PNH) requires a heightened index of suspicion, and once confirmed, patients with haemolytic PNH and/or PNH with thrombosis should be considered for anti-complement therapy.
- Eculizumab is the only approved therapy for PNH in Singapore; other complement inhibitors are under various stages of investigation.

Clinical Implication

- This expert consensus will help guide healthcare professionals to optimise the screening, diagnosis and management of PNH in Singapore.

INTRODUCTION

Paroxysmal nocturnal haemoglobinuria (PNH) is a rare clonal blood disorder caused by acquired mutations in haematopoietic stem cells.[1] Mutations in the X-linked phosphatidylinositol glycan class A (PIG-A) gene and consequent impairment in glycosylphosphatidylinositol (GPI) anchor synthesis result in the deficiency of complement-inhibiting proteins, CD55 (decay accelerating factor) and CD59 (membrane inhibitor of reactive lysis).[1,2] The deficiency of CD55 and CD59 in red blood cells (RBCs) results in an increased sensitivity of erythrocytes to complement-mediated intravascular haemolysis (IVH), lysis of RBCs and release of haemoglobin into plasma, which may lead to haemoglobinuria.[1]

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Clinically, PNH is characterised by IVH, increased susceptibility to thrombosis and bone marrow failure.[3] While IVH is the hallmark feature of PNH,[3] the deficiency of nitric oxide (NO) due to its sequestration by free plasma haemoglobin may increase the risk of thrombotic events that may affect multiple organs, including the liver, kidneys, central nervous system and/or lungs.[3,4] Other disease sequelae include renal failure and pulmonary hypertension.[5] Common PNH symptoms include dark-coloured urine (observed in 25% of patients), fatigue, anaemia and shortness of breath.[3,6] Additional non-specific signs related to NO depletion (and vasoconstriction) include abdominal pain, chest pain,[7] dysphagia and erectile dysfunction in men.[7]

Globally, PNH incidence is 1–1.5 cases per million individuals.[8] However, there is limited data on its prevalence in Singapore. PNH affects both men and women equally, but a few studies suggest a slightly high prevalence in women due to lyonisation.[8] Clinical manifestations of PNH can occur across all ages, with rare cases in children and a higher incidence between 30–40 years.[9] PNH may be associated with aplastic anaemia (AA), myelodysplastic syndromes (MDS) or acute myeloid leukaemia (AML). In most cases, PNH develops in patients previously diagnosed with AA or MDS.[2] The disease burden of PNH varies widely and is influenced by the proportion of GPI-anchor protein (AP) deficient cells or clone size.[3] Despite the most comprehensive supportive care, PNH may be associated with a 35% mortality rate at 5 years and approximately 50% at 10 years.[5]

The International PNH Interest Group classifies PNH into 3 subgroups: (1) classic PNH, characterised by clinical and laboratory findings of IVH without evidence of bone marrow deficiency; (2) PNH associated with bone marrow disorder (e.g. AA, MDS); and (3) subclinical PNH, observed in patients with a small population of PNH cells, without evidence of haemolysis or thrombosis.[5]

While international guidelines exist,[4-7,10-13] clear recommendations for PNH screening, diagnosis and management in Singapore are lacking. This, in addition to the lack of awareness among general healthcare professionals about various manifestations of PNH, may hinder timely referral, diagnosis and treatment initiation. Moreover, limited access and funding for complement inhibitors, such as eculizumab, may potentially limit treatment options, affecting outcomes in patients with PNH. Therefore, we used a modified Delphi methodology to develop evidence-based recommendations for screening, diagnosing and treating subclinical and classic PNH to optimise disease management and facilitate improved access to emerging treatments in Singapore.

METHOD

Expert panel

Nine haematologists from different academic institutions and private medical centres across Singapore were identified based on their clinical expertise in treating PNH and relevant publication experience. An expert haematologist from Taiwan, involved in developing the Taiwanese PNH guidelines, was also invited to share his clinical insights.

Literature search, review and evidence rating

Seven core categories focusing on the key aspects of PNH screening, diagnosis and management were identified, and clinical research questions were drafted for each category: (1) indications for screening of PNH; (2) diagnosis of PNH; (3) clinical, laboratory and imaging investigations after the diagnosis of PNH; (4) treatment and monitoring of patients with subclinical PNH; (5) treatment and monitoring of patients with classic PNH; (6) treatment and monitoring of patients with PNH in the setting of underlying bone marrow disorders; and (7) treatment and monitoring of patients with PNH in pregnancy.

A comprehensive literature search was performed in Medline via PubMed using search strings developed from a combination of relevant medical subject headings and free-text terms. Studies (randomised controlled trials [RCTs], observational studies, systematic reviews and meta-analyses) spanning January 2010 through July 2023, conducted in humans, with abstracts in English, were considered for inclusion. As eculizumab was approved in 2007, all articles from January 2006 to December 2009 on the use of eculizumab for the treatment of PNH were also included in the initial screening. Narrative reviews, news articles, letters to the editor and commentaries were excluded. The search results were screened by title and abstract, followed by a full-text review of shortlisted articles and data extraction for the final set. Clinical guidelines for PNH screening, diagnosis and management were also considered and reviewed while preparing the statements (Supplementary Fig. S1).

Through an iterative editing process, draft statements were developed using available evidence and real-world clinical experience from the core expert panel. The quality of evidence

supporting the PNH management recommendations was assessed using the Oxford Levels of Evidence 2011 (Oxford Centre for Evidence-Based Medicine, Supplementary Table S1).[14]

Consensus building

The consensus recommendations were developed by a modified Delphi-based approach, described by Gustafson et al.[15] The draft statements underwent the first round of Delphi voting (August 2023) using Microsoft Forms. Experts independently rated statements on a Likert scale (1: completely agree to 5: completely disagree) with an option to submit their comments for further optimisation. Consensus (the average of ratings for "completely agree" and "agree with minor changes") was set a priori at ≥70% agreement.[16] Delphi round 1 results and statements were presented and discussed in an advisory board meeting on 13 September 2023, where the expert panel discussed to further refine the statements based on real-world practices in Singapore. Revised draft statements were again shared for final agreement rating (October 2023). The strength of the consensus was defined as "strong" (>90% agreement), "moderate" (70–90% agreement) and "no consensus" (<70% agreement).

RESULTS

The systematic literature search identified 1679 records. After title and abstract screening, and deduplication, 290 articles underwent detailed full-text screening. Finally, 181 articles on PNH diagnosis (n=48) and treatment (n=133) were considered for data extraction (Supplementary Fig. S1) to draft 16 consensus statements and substatements. Additionally, 10 international and regional PNH guidelines were reviewed to optimise the statements. While the key studies are cited in the paper, additional articles that were reviewed to develop the consensus statements are included in Supplementary Table S2. Over 80% of included studies were observational, and draft statements on PNH management were primarily supported by level 2 evidence. Nevertheless, all statements achieved >90% agreement (strong recommendation) after 2 rounds of Delphi voting (Supplementary Table S3).

DISCUSSION

When to consider PNH?

Early identification of a PNH defect is crucial for optimising the treatment and improving the prognosis of the disease. Although PNH is characterised by a triad of haemolysis, thrombosis and bone marrow failure, patients can present with varying combinations of symptoms. The most common initial presenting signs and symptoms include anaemia[17] (severe fatigue,[18] dyspnoea,[18] headache[17]) and/or dark-coloured urine.[19] The NO deficiency in PNH often leads to gastrointestinal muscular dystonia, manifested by abdominal pain, and vascular muscular dystonia, resulting in dysphagia,[17] chest pain[7] and erectile dysfunction.[17] The non-specific nature of these symptoms can lead to delays in diagnosis and treatment. To facilitate more efficient screening of PNH, the experts proposed that 3 groups of patients should be considered for further evaluation for the diagnosis of PNH (Fig. 1, Statement 1).

The first group includes patients with Coombs' negative haemolytic anaemia[4,6,12] and one or more of the following features, based on observational studies: haemoglobinuria,[17] renal dysfunction,[17] elevated serum lactate dehydrogenase (LDH, ≥1.5 x upper limit of normal [ULN]),[17] elevated reticulocyte count,[19] reduced serum haptoglobin[19] or unexplained iron deficiency.[17] Chronic IVH may result in renal disease, and the presence of unexplained haemolysis with signs of renal failure may indicate the need for further PNH testing.

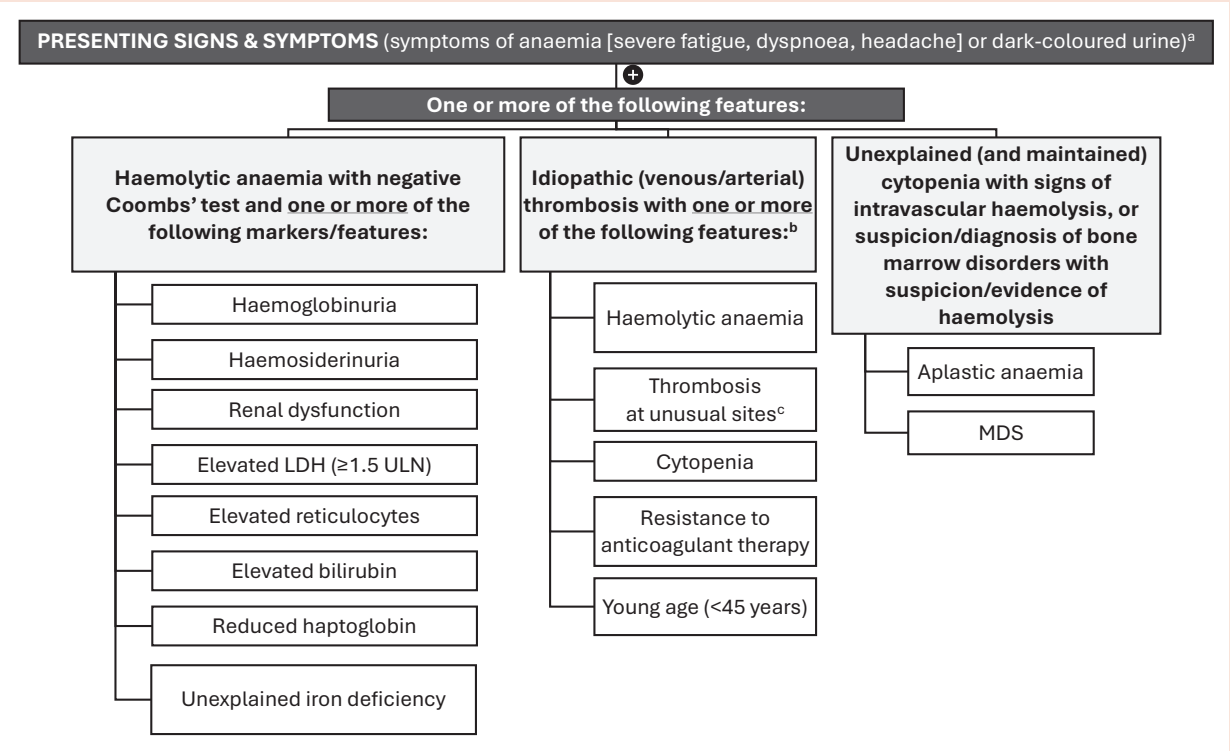
The second group includes patients with unexplained or unusual sites of thrombosis, possibly with signs of intravascular haemolysis or cytopenia. Patients experiencing thrombosis despite adequate anticoagulation therapy, especially young patients (<45 years),[17] should also be tested for PNH. About 29–44% of patients report thromboembolic events at least once in the course of the disease, with these events being the main cause of death in 40–67% of cases before the introduction of eculizumab treatment.[20] Some of the common thrombosis sites in PNH include cerebral and intra-abdominal regions (including portal[17] and hepatic veins, resulting in Budd–Chiari syndrome).

The third group includes patients with evidence of bone marrow dysfunction, including AA and MDS, coupled with suspicion or signs of haemolysis. Cytopenia, a consequence of bone marrow failure (BMF) syndrome, is often present concomitantly with subclinical PNH.[21] While IVH is the main cause of haemolytic anaemia in PNH, the underlying anaemia may be aggravated by BMF. Abnormal erythropoiesis is reported in cases of haemolysis associated with BMF.[22] Throughout the course of PNH, BMF of varying severity is detected in almost all patients.[23] In classic PNH, approximately 30–40% of patients develop AA, MDS or AML during a 10-year follow-up.[22]

Diagnosis of PNH

Flow cytometry is the gold standard for the diagnosis of PNH (Table 1, Statement 2). The

Fig. 1. Indications to consider screening of PNH (Statement 1).



LDH: lactate dehydrogenase; MDS: myelodysplastic syndrome; PNH: paroxysmal nocturnal haemoglobinuria; ULN: upper limit of normal
[a] Other clinical features may include intermittent dysphagia, abdominal pain or erectile dysfunction of unknown aetiology
[b] Arranged in an order with the subcriterion raising the highest suspicion of PNH at the top. Young age as the sole subcriterion is not sufficient to raise the suspicion for PNH in patients with idiopathic thrombosis and should be considered along with any one of the other listed subcriteria, based on clinical discretion and on a case-to-case basis.
[c] Hepatic veins (Budd–Chiari syndrome), cerebral venous sinus, cutaneous veins and other intra-abdominal veins (portal, splenic, visceral)

use of the latest International Clinical Cytometry Society/European Society for Clinical Cell Analysis recommendations not only helps detect GPI-defective cell clones in PNH but also identifies even fewer cells with the PNH phenotype in patients with BMF.[24] Tests are routinely performed on neutrophils, monocytes and erythrocytes from peripheral blood. GPI-defective cell identification is performed by labelling cells with monoclonal antibodies against GPI-AP antigens or fluorescently labelled aerolysin (FLAER), which directly binds to GPI anchors.[17]

The diagnostic criteria mandate the demonstration of deficiencies in at least 2 distinct GPI proteins within 2 separate cell lines—granulocytes, monocytes or erythrocytes—via flow cytometry.[6] The diagnosis of PNH is confirmed in individuals who meet the following criteria: (1) granulocytes: absent or decreased expression of FLAER or CD24, (2) monocytes: absent or decreased expression of FLAER or CD14, and (3) red cells: absent or decreased CD59 expression (type II and type III PNH populations).[22] The flow cytometry tests have the capability to quantify the proportion

Table 1. Recommendations for optimising the diagnosis of PNH (Statement 2).

Statement 2.1	We recommend the use of FLAER or high-sensitivity flow cytometry to detect the deficiency of GPI-anchored proteins in peripheral blood (leucocytes [both neutrophils and monocytes] and erythrocytes), to confirm the diagnosis of PNH.[a]
Statement 2.2	Annual follow-up flow cytometry may be considered when clinically indicated in patients with clone size <1% on initial flow cytometry. While 6-monthly follow-ups may be considered in patients with (1) clone size >1% on initial flow cytometry or (2) underlying bone marrow failure syndromes, especially in case of disease progression or for guiding treatment.[b]

FLAER: fluorescently labelled aerolysin; GPI: glycosylphosphatidylinositol; PNH: paroxysmal nocturnal haemoglobinuria
[a] Based on the International Clinical Cytometry Society guidelines to detect GPI-deficient cells in PNH and related disorders
[b] Subject to clinician's discretion on a case-to-case basis

of cells exhibiting the PNH abnormality. Assessing the size of the PNH clone within the neutrophil or monocyte population provides the most accurate reflection of disease progression, as the circulation of PNH erythrocytes (type II and type III) is generally low. Normal erythrocytes (type I), which are CD59-positive, have an extended lifespan of approximately 120 days. Conversely, erythrocytes with diminished CD59 expression (type II) are 3 to 5 times more sensitive to complement attack. CD59-negative red cells (type III) are markedly more vulnerable and 15 to 25 times more susceptible to complement attack than type I cells. Consequently, type III cells have a shortened circulating lifespan of 10 to 15 days as they are swiftly eliminated from circulation, especially during periods of complement system activation such as infections.[22]

Laboratory investigations after confirmation of PNH diagnosis

Comprehensive laboratory and imaging investigations should be conducted in patients diagnosed with PNH, in addition to the clinical evaluation described under screening. Full blood and reticulocyte count, as well as serum LDH, haptoglobin and bilirubin levels, should be assessed to confirm IVH.[4-7] Haemoglobin concentration and serum haptoglobin levels are low, while reticulocyte count, serum LDH and bilirubin levels are elevated in patients with PNH. The diagnosis may be further confirmed by the detection of haemosiderin in urine through microscopy and a urine dipstick test.[4-7] Other investigations include testing for D-dimer, prothrombin time, activated partial thromboplastin time and international normalised ratio.[5] Renal function should be assessed by analysing serum creatinine levels and proteinuria.[11] The absence of antibody-mediated IVH in PNH results in a negative direct antiglobulin (Coombs) test in treatment-naïve patients (Table 2, Statement 3).

Imaging investigations include computed tomography or ultrasound for thromboembolic events, echocardiogram for pulmonary hypertension, and cranial magnetic resonance imaging for intracranial thrombosis in case of headache or other neurological symptoms. A bone marrow aspirate or biopsy is recommended for patients with suspected concomitant AA/MDS.[4,6,7,11] A summary of clinical, laboratory and imaging assessments for confirmed PNH diagnosis, based on existing guidelines[4,6,7,11] and real-world practices in Singapore, is outlined in Table 2 (Statement 3).

Management of PNH

Subclinical PNH

The expert panel recommendations for optimising the management and monitoring of subclinical PNH in Singapore are shown in Table 3 (Statement 4). As subclinical PNH is asymptomatic with a low thromboembolic risk compared to classic PNH, no treatment is needed.[3,5,9] In subclinical PNH, blood cells lack GPI-AP and the PNH clone size (≤10% granulocyte clone) is typically small, often significant only in the context of immune-mediated BMFs.[9] Therefore, the primary focus of treatment should be on addressing the underlying bone marrow disease—AA (in PNH/AA) or MDS (in PNH/MDS).[5] However, close monitoring every 6–12 months or more (in stable cases) may be considered as progression to haemolytic or thrombotic PNH may occur due to PNH clone expansion or the emergence of cells with a PNH phenotype.[9,11]

Classic PNH

Classic PNH, diagnosed in approximately one-third of patients, is characterised by normocellular to hypercellular bone marrow, erythroid hyperplasia, elevated reticulocyte count and LDH levels 2–10 times ULN.[9] It usually features large PNH clones (mean granulocyte clone size of >50),[22] with thrombosis risk proportionate to clone size.[5] Other manifestations include smooth muscle dystonia, fatigue, renal impairment and pulmonary hypertension.[9]

Six recommendations were drafted by the expert panel for the management of classic PNH in Singapore and are provided in Supplementary Fig. S2 (Statements 5.1–5.8), Table 3 (Statements 5.2–5.4 and 5.6) and Table 4 (Statement 5.5). Multidisciplinary clinical management involves optimal management of haemolysis with standard-of-care complement C5 inhibitors (e.g. eculizumab) and tailored supportive treatments for symptomatic anaemia, haemoglobinuria, iron deficiency, thrombosis, smooth muscle dystonia, kidney damage and/or underlying BMF disorders such as AA and MDS (Supplementary Fig. S2, Statement 5.1).

Complement inhibitor therapy

Complement inhibitors are indicated for classic haemolytic PNH, and criteria for initiating complement C5 inhibitors are detailed in Table 3 (Statement 5.2). These indications are in line with the existing guidelines.[6,7,22] Eculizumab, the first monoclonal antibody targeting complement C5

Table 2. Clinical and laboratory/imaging investigations after the confirmation of PNH diagnosis (Statement 3).

Investigations	**Purpose/interpretation**
Clinical history	
Severe asthenia, dyspnoea	Anaemia
Dark-coloured urine, jaundice	Intravascular haemolysis
Oesophageal spasm, dysphagia, abdominal pain, erectile dysfunction (unexplained)	Smooth muscle dysfunction
Abdominal pain, chronic headache, neurological deficit	Atypical thrombosis
History of fever/infections	Granulocytopenia
Co-existing bone marrow failure syndromes (AA/MDS)	To aid in treatment planning
Medication and transfusion history	
Pregnancy history and/or plans	
Baseline investigations	
Complete blood count with haemoglobin, neutrophils, platelets	Low levels indicate anaemia or cytopenia (granulocytopenia or thrombocytopenia)
Reticulocyte count	Elevated count indicates haemolysis
Serum LDH	≥1.5 x ULN indicates haemolysis
Unconjugated or indirect bilirubin	Elevated levels indicate haemolytic anaemia
Haptoglobin	Low levels indicate intravascular haemolysis
Serum iron profile (ferritin, total iron binding capacity)	Low ferritin and high total iron binding capacity indicate iron deficiency
Serum creatinine	High levels indicate renal insufficiency
Prothrombin time (PT)	To guide anticoagulant therapy or thromboprophylaxis
Activated partial thromboplastin time (aPTT)	
Direct antiglobulin (Coombs') test	Negative test excludes autoimmune haemolytic anaemia
Urine dipstick	Clear, red/amber-coloured urine that remains pigmented (with haemoglobin) after centrifugation, with haemoglobin in urine indicates haemoglobinuria during haemolytic episodes
Urine microscopy	Presence of stainable iron indicates intravascular haemolysis and haemosiderinuria
Urinary albumin	Presence (above reference) indicates microalbuminuria and proteinuria
Glomerular filtration rate	Low levels (below reference) indicate renal dysfunction
Bone marrow aspirate (cytology and cytogenetics)	In patients with suspected or concomitant AA/MDS
Bone marrow biopsy	
Investigations (only when clinically indicated)	
Serum erythropoietin	Levels are often high in PNH and correlate with reticulocyte count
Abdominal ultrasound with Doppler	To detect thrombi
Doppler echocardiography	To evaluate pulmonary hypertension
Pulmonary CT angiography	To evaluate pulmonary hypertension
Cranial MRI/CT	Relevant in case of headache or other neurological symptoms

Table 2. Clinical and laboratory/imaging investigations after the confirmation of PNH diagnosis (Statement 3). (Cont'd)

Investigations	Purpose/Interpretation
Investigations (only when clinically indicated)	
Bone density	Relevant in patients on prior steroids
Abdominal MRI	To assess the degree of hepatic iron deposit
Investigations (optional)	
HLA typing	May be considered in young patients for future stem cell transplantation
NT-proBNP	In patients with pulmonary hypertension (higher than reference levels indicate heart failure)
Vitamin B12	To exclude other causes of anaemia
Folic acid	

AA: aplastic anaemia; CT: computed tomography; HLA: human leucocyte antigen; LDH: lactate dehydrogenase; MDS: myelodysplastic syndromes; MRI: magnetic resonance imaging; NT-proBNP: N-terminal pro-B-type natriuretic peptide; PNH: paroxysmal nocturnal haemoglobinuria; ULN: upper limit of normal

protein, is approved for the treatment of PNH, with subsequent Food and Drug Administration (FDA) and European Medicines Agency (EMA) approval for ravulizumab. Both inhibitors block terminal complement C5 activation, preventing C5a and C5b-9 formation.[5] As of August 2023, only eculizumab is approved in Singapore, based on the results of 2 large phase III randomised studies, TRIUMPH (87 cases)[25] and SHEPHERD (97 cases).[26] The studies demonstrated that eculizumab prevents IVH in PNH, eventually leading to haemoglobin stabilisation; reduction or elimination of the need for RBC transfusions; and resolution of most disease-related symptoms. A follow-up study showed a substantial reduction in the rate of thromboembolic events, dropping from 7.4 to 1.1 events per 100 patient-years.[27] A systematic review of 6 studies confirmed eculizumab's efficacy in decreasing LDH levels and transfusion rates and in increasing haemoglobin levels.[28] Long-term follow-up studies (up to 5 years) have confirmed the efficacy of continuous maintenance eculizumab treatment with haematological improvement and no safety concerns.[29] Eculizumab also reduced thromboembolic risk (relative risk reduction: 85%) over 3 years, thus reducing morbidity and improving long-term survival in patients with PNH.[27]

In an open-label phase II study, AEGIS, involving 29 Japanese patients with PNH, eculizumab significantly reduced haemolysis (87%, $P<0.0001$) and transfusion frequency ($P=0.006$).[30] Fatigue and dyspnoea significantly improved within 1–2 weeks of treatment, independent of changes in haemoglobin.[30] These results were confirmed in a 2-year long-term and post-marketing surveillance study in Japanese patients, demonstrating sustained reduction in IVH ($P<0.001$) and RBC transfusions ($P=0.0016$) compared with baseline levels.[31] Two independent studies also showed >90% 5-year survival rates for patients with PNH who received continuous treatment with eculizumab.[32,33] A study by Ueda et al. showed improved quality of life (QOL) patients with PNH receiving eculizumab,[34] revealing a relationship between the QOL test components and haemoglobin and LDH concentrations.[34] The safety and efficacy of eculizumab have also been demonstrated in paediatric patients.[35]

Ravulizumab is indicated for treating PNH in adults and children (≥10 kg) with haemolysis and clinical symptom(s) indicative of high disease activity, and who are clinically stable after ≥6 months of previous eculizumab treatment.[36] Ravulizumab has a mean terminal half-life approximately 4 times longer than eculizumab, providing complete and sustained terminal C5 inhibition with an 8-week dosing interval.[36] Two studies (phase Ib and phase II) and 2 large phase III trials (n=441 for both) evaluated the efficacy and safety of ravulizumab; one phase III study was in eculizumab-treated patients (study 302) and the other in treatment-naïve PNH patients (study 301).[37-39] Results revealed that ravulizumab was non-inferior to eculizumab for haemolysis control and transfusion avoidance, with similar adverse events.[40] These results were maintained in long-term studies.[41,42] In a long-term study spanning from 27 weeks to 2 years and involving over 400 patients with PNH previously (662 patient-years), ravulizumab sustained improvement in LDH levels in both study populations (studies 301 and 302). The study reported that 81.9% of patients in study

Table 3. Recommendations for the management of subclinical PNH (Statement 4) and classic PNH (Statement 5).

Management of subclinical PNH		
Statement 4.1	Asymptomatic patients with subclinical PNH with small PNH clones and no evidence of haemolysis may be monitored every 6–12 months for (1) symptoms of haemolysis, underlying bone marrow disorder and emerging complications, and (2) expansion or evolution of subclinical PNH clones by FLAER or high-sensitivity flow cytometry.[a]	
Statement 4.2	Initiation of anti-complement therapy is not needed in patients with subclinical PNH. Appropriate treatment should be offered to treat the underlying bone marrow disease and associated complications, and thromboprophylaxis may be initiated in patients at high risk of thrombosis (e.g. during pregnancy).	
Management of classic PNH		**Level of evidence**
Statement 5.2	Complement C5 inhibitors are indicated for the treatment of patients with PNH, with increased haemolysis (LDH >1.5 ULN), granulocyte PNH clone >10%, and one or more of the following criteria: • clinical symptoms indicative of high disease activity (weakness, fatigue, haemoglobinuria, abdominal pain, dyspnoea, anaemia [Hb <10 g/dL], thrombosis, dysphagia and/or erectile dysfunction), regardless of transfusion history • history of thromboembolic events requiring anticoagulant therapy due to PNH • history of regular transfusions (at least 4 packs of RBC over the past 12 months) due to haemolysis • organ damage due to haemolysis (chronic renal failure or repeated episodes of acute renal failure; chest pain with New York Heart Association class III or IV; respiratory failure or an established diagnosis of pulmonary hypertension; and/or smooth muscle dystonia) • pregnancy with a high risk of thrombosis or history of gestational complications	2
Statement 5.3.1	Consider (1) increasing the dose of eculizumab, (2) decreasing the time between infusions or (3) switch-over from eculizumab to ravulizumab,[b] in case of inadequate response to eculizumab therapy (transfusion dependency due to intravascular haemolysis or failure to decrease serum LDH levels to <1.5 x ULN) or regular breakthrough haemolysis during eculizumab treatment (≥3 months).[c]	2
Statement 5.3.2	Switch-over from C5 to C3 inhibitor therapy[d] may be considered in case of any one of the following conditions: (1) breakthrough intravascular haemolysis during regular C5 inhibitor treatment (≥3 months) (2) clinically relevant C3-mediated extravascular haemolysis on C5 inhibitor treatment (≥3 months) (3) unprovoked thromboembolic event during C5 inhibitor therapy (4) unexplained severe fatigue and impaired quality of life despite C5 inhibitor therapy for ≥3 months (5) inadequate response to C5 inhibitor therapy (transfusion dependency due to intravascular haemolysis or failure to decrease serum LDH levels to <1.5 x ULN)	2
Statement 5.4	Vaccination against meningococcus with a tetravalent vaccine including serotypes A, C, Y and W135, along with vaccination against serotype B[e] is recommended at least 2 weeks before initiating treatment with C5 inhibitor therapy.	2
Statement 5.6	Complement C5 inhibitor therapy should ideally be continued for an extended duration. Discontinuation of treatment may be considered in selected cases with significant lack of clinical improvement, severe bone marrow failure, non-compliance/contraindications to treatment or due to patient's decision to stop the treatment.	2

FLAER: fluorescently labelled aerolysin; Hb: haemoglobin; LDH: lactate dehydrogenase; PNH: paroxysmal nocturnal haemoglobinuria; RBC: red blood cell; ULN: upper limit of normal
[a] Monitoring using FLAER or flow cytometry may be considered on a case-to-case basis
[b] Subject to local approval, patient factors and resource constraints
[c] Exclude acute or pharmacodynamic causes such as infections or pregnancy
[d] Subject to local approval, patient factors and resource constraints
[e] Subject to availability

301 and 85.6% of patients in study 302 maintained transfusion avoidance. Additionally, FACIT-F scores remained stable.[42] Similar results were reported in a sub-analysis of Japanese patients (study 301: n=33; study 302: n=12), which evaluated the efficacy of ravulizumab in PNH,[43] with 83.3% (15 of 18) of patients successfully avoiding transfusion (study 301) and with adjusted prevalence of 52.1% for LDH normalisation. In study 302, the least-squares-mean percentage change from baseline in LDH was 8.34%.[43]

All individuals with large PNH clones (>50% granulocytes, >10% erythrocytes) significantly elevated LDH levels, and a high reticulocyte count may benefit from eculizumab therapy,[22] but only a fraction with high disease activity show

Table 4. Recommended follow-up assessments in patients with PNH along with their frequency (Statement 5.5).

Follow-up investigations	Frequency[a]
Clinical symptoms (fatigue, pain [abdominal pain, oesophageal spasms], episodes of increased haemolysis, haemoglobinuria, anaemia, dyspnoea, erectile dysfunction)	Monthly for 3 months after initiating therapy for PNH followed by every 3 months
CBC, reticulocyte count, LDH	
Renal function (electrolytes, estimated creatinine clearance, microalbumin, urine analysis [routine and microscopic])	
Liver function test	
Iron status (ferritin, transferrin saturation)[b]	Every 3 months
Direct Coombs' test[c]	Every 3–6 months
NT-proBNP	Every 6 months
History of transfusions	Every 6 months
FLAER or high-sensitivity flow cytometry (PNH clone analysis)	As per Statement 2.2
Meningococcal infection and history of penicillin or antibiotics for meningococcal prophylaxis[d]	Every 12 months
MRI to analyse hepatic iron deposition (in case of iron overload)[b]	
2D echocardiography[b]	

CBC: complete blood count; FLAER: fluorescently labelled aerolysin; LDH: lactate dehydrogenase; MRI: magnetic resonance imaging; NT-proBNP: N-terminal pro-B-type natriuretic peptide; PNH: paroxysmal nocturnal haemoglobinuria
[a] Frequency of follow-up may be revised at the clinician's discretion based on disease activity
[b] Only when clinically indicated
[c] In patients with suspected autoimmune haemolytic anaemia
[d] Only in patients on complement C5 inhibitor therapy

significant improvement in haemoglobin levels and become transfusion-free.[44] Possible reasons for this lack of desired treatment response include (1) very rare C5 polymorphisms,[45] mainly observed in Japanese patients, hindering eculizumab (and ravulizumab) binding at desired epitopes; (2) suboptimal eculizumab dosing leading to recurrent pharmacokinetic breakthrough haemolysis (BTH);[46,47] (3) pharmacodynamic BTH associated with complement activity leading to infections or inflammation;[48] and (4) eculizumab (or C5 inhibitors) causing C3-fragment associated extravascular haemolysis (EVH).[48] BTH occurs in 11–27% of patients receiving eculizumab.[46] Once BTH is confirmed, management options include increasing eculizumab dose (e.g. 1200 mg every 14 days) or shortening infusion intervals (e.g. every 12 days) (Table 3, Statement 5.3.1). Another strategy is switching from eculizumab to ravulizumab, as ravulizumab may prevent BTH by keeping free C5 levels lower than eculizumab.[37,41,49-51] An open-label, randomised, phase III study in eculizumab-treated adult patients with PNH who switched to ravulizumab demonstrated the non-inferiority of ravulizumab to eculizumab in efficacy and safety.[52]

In a US cross-sectional study in patients with PNH on eculizumab (n=35) or ravulizumab (n=87), approximately 85% were anaemic, 80% experienced fatigue and 10–20% had thromboembolic events despite 12 months of C5-inhibitor treatment.[53] Similar results were reported in a recent US-based electronic medical record network study; after 12 months of treatment with C5 inhibitor, 50–82% remained anaemic, 8–32% required ≥1 transfusion and 13–59% had BTH.[54] This highlights an unmet need for newer treatments for PNH with better outcomes. Agents targeting complement C3 (e.g. pegcetacoplan, approved in 2021 by FDA and EMA) are underway. These agents primarily prevent EVH caused by the activation of complement C3.[22] Approval of pegcetacoplan was based on the results of a 16-week, multicentre, randomised, open-label, active comparator-controlled phase III clinical trial, PEGASUS. The results revealed that pegcetacoplan was superior to eculizumab in enhancing haemoglobin levels with an adjusted (least squares) mean difference of 3.84 g/dL (P<0.001) and non-inferior in various clinical and haematologic outcomes.[55] A systematic review of 3 studies showed that pegcetacoplan effectively improves haemoglobin level and decreases transfusion requirements in patients with PNH, including those unresponsive to eculizumab.[56] Over a 48-week treatment period,

pegcetacoplan exhibited superior long-term efficacy and safety compared to eculizumab. There was a statistically significant improvement in adjusted mean haemoglobin level (3.8 g/dL) at week 16 (P<0.001). Moreover, 85% of pegcetacoplan-treated patients were transfusion-free, whereas only 15% of eculizumab-treated patients achieved this outcome.[57] However, during the study period, 7% (3 of 41) pegcetacoplan-treated patients discontinued treatment due to BTH. In other studies, pegcetacoplan showed improved QOL after 26 weeks in complement inhibitionnaïve PNH patients.[58,59] The expert panel recommends the criteria listed in Table 3, Statement 5.3.2, for switching from C5 to C3 anti-complement therapy.

Infection is a major risk with complement inhibitors.[22] Eculizumab increases the risk of life-threatening infections with *Neisseria* spp., including *N. meningitidis*, by blocking terminal complement activation.[2,52] The estimated risk is 0.5% per year or 5% after 10 years of treatment.[2] Therefore, all patients with PNH planned for eculizumab initiation should be vaccinated against *Neisseria* with a quadrivalent vaccine against ACYW135 serotypes and serogroup B (subject to availability) at least 2 weeks before the first dose of eculizumab (Table 3, Statement 5.4).[4-6,10,60]

Appropriate monitoring is important for assessing treatment response and overall disease outcome, and for predicting plausible risks. The expert panel recommends monthly assessments of full blood and reticulocyte count, serum LDH and bilirubin for patients on eculizumab during the initial 3 months, followed by 3-monthly intervals[4,5,7,11] (Table 4, Statement 5.5). Reassessing the benefits of eculizumab every 6 months, based on the patient's clinical progress and laboratory results, is advisable. Treatment discontinuation may be considered in non-adherent patients after a comprehensive assessment and weighing the pros and cons[7] (Table 3, Statement 5.6).

Allogenic stem cell transplantation

Allogenic stem cell transplantation (SCT) still remains the only curative treatment for PNH; however, it is associated with significant morbidity and mortality. The most extensive study on SCT in PNH was conducted at French centres between 1978 and 2007. Out of the 211 patients with PNH, 62% underwent transplantation for BMF, 70% for haemolysis and 25% for thromboembolism.[61] At 5 years, 40% experienced Grades 2–4 acute graft-versus-host disease (GvHD), and 29% had chronic GvHD. Overall survival (OS) was 68%, with varying rates based on indication: highest for haemolysis (86%), followed by BMF (69%) and thromboembolism (54%).[61]

The morbidity and mortality outcomes associated with SCT have improved considerably in the post-eculizumab era. A retrospective analysis of 78 patients with PNH (27 and 51 patients with type I and type II PNH, respectively) transplanted between 2002 and 2016 in 11 centres of the Polish Adult Leukemia Group showed a 3-year OS of 87% in the total cohort and 92% in the group of patients without thrombosis.[62,63] While the survival of PNH patients with SCT has improved over time, long-term post-transplant complications such as GvHD will likely result in less favourable QOL compared to eculizumab.[64] Hence, SCT is not recommended as an initial therapy and should be limited to selected young patients with PNH/AA or PNH/MDS (Supplementary Fig. S2, Statement 5.8).[65]

Anticoagulation therapy

In patients with a large PNH clone who are not receiving complement inhibitors (eculizumab), primary prophylaxis with anticoagulant therapy should be considered to reduce the risk of thrombosis, if there is no contraindication such as thrombocytopenia or bleeding risk.[6,22] These patients remain at a high risk of thrombotic events and death, despite anticoagulation treatment,[66] underscoring the need to initiate eculizumab in this population. On the other hand, primary prophylaxis may be discontinued once complement inhibitors are initiated, as it may offer little benefit and increase the risk of bleeding complication[22] (Supplementary Fig. S2, Statement 5.7).

In PNH patients with a history of thromboembolic events who have been initiated on complement inhibitors, long-term anticoagulation (with coumarin derivatives and heparin) is recommended by several guidelines.[4,66] The decision to stop anticoagulation in these patients has to be individualised. Emerging evidence and expert opinion suggest that anticoagulation for 3–6 months is sufficient in PNH patients who are well-controlled on complement inhibitors.[67,68] Extended duration (lifetime) anticoagulation can be considered in patients with additional provoking risk factors for thrombosis and those with a history of life-threatening thrombotic events.

Supportive therapy

Supportive therapy plays an important role in treating the symptoms and complications of PNH.[1,6,7,10] Folic acid, vitamin B12 and packed RBC transfusions are provided in the presence of symptomatic anaemia and haemoglobinuria. Oral or parenteral iron supplementation is initiated to prevent and control iron deficiency from haemoglobinuria and haemosiderinuria. Short-term steroids may be considered in haemolytic episodes;

long-term treatment is not recommended. Analgesia can be considered for smooth muscle dystonia.

PNH in the setting of BMF

The expert panel recommends that patients with small PNH clones in the context of BMF syndromes may be assessed every 6–12 months (Table 5, Statement 6.1). The beneficial effects of eculizumab treatment in patients with PNH/AA or PNH/MDS without haemolysis or thrombosis have not been well established.[69] However, patients with AA or MDS and a high percentage of PNH cells may benefit from the use of C5 inhibitors. In individuals with predominant BMF, immunosuppressive therapy and/or SCT should be considered (Table 5, Statement 6.2).[6]

PNH in pregnancy

There is a high risk of thrombosis during pregnancy, thus increasing maternal-fetal morbidity and mortality. Studies have suggested that the maternal mortality rate during pregnancy and shortly after childbirth is estimated to be 12–21% in patients with PNH.[70,71] Additionally, there is a high risk of experiencing miscarriages or premature births. No RCTs have been conducted to evaluate the use of eculizumab or ravulizumab in pregnant females. Based on the findings from observational studies, the panel recommends that pregnant women with PNH may be treated with eculizumab to prevent the risk of thromboembolic complications. Eculizumab is reported to be safe in pregnancy, with no untoward effects on the mother and the child (Table 5, Statement 7.1).[70] Pregnant women should be monitored frequently, as there may be a need for higher doses of eculizumab to mitigate haemolysis and minimise the risk of thrombotic events (Table 5, Statement 7.3). An analysis of 75 pregnancies involving 61 women with PNH showed that eculizumab reduced the rate of maternal complications and improved the rate of fetal survival.[70] The recommendation in Statement 7.2 (Table 5) on prophylactic or therapeutic anticoagulation in pregnant women with PNH is in line with the existing guidelines.[4-7] As the risk of thrombosis is usually high in most cases for at least 6 weeks post-partum, anticoagulants can be extended at the clinician's discretion on a case-to-case basis.[4,70]

Novel treatment options for PNH

Several novel anti-complement agents for PNH are in various stages of clinical development, including crovalimab (phase III studies are underway), tesidolumab (LFG316), pozelimab, zilucoplan and cemdisiran.

Crovalimab, a sequential, highly soluble, monoclonal antibody recycling technology (SMART) antibody

Table 5. Management of PNH in special populations (Statements 6 and 7).

In the setting of BMF		**Level of evidence**
Statement 6.1	Patients with small PNH clones in the context of bone marrow failure syndromes may be assessed every 6–12 months with flow cytometry for expansion or evolution of PNH clones, especially when there is evidence of haemolysis.	2
Statement 6.2	The use of immunosuppressants or allogeneic haematopoietic stem cell transplantation may be considered in patients with PNH (non-pregnant) and associated bone marrow disorders such as AA or high-risk MDS, based on the risk/benefit profiles of the treatments. The underlying bone marrow failure may be treated as per the respective treatment (AA/MDS) guidelines. Additional supportive therapy may be based on the corresponding recommendations for classic PNH.	2
In pregnancy		
Statement 7.1	Eculizumab treatment may be continued in pregnant women with PNH, especially those with associated risk factors for thrombosis. The dose of eculizumab may be increased in the third trimester or in case of breakthrough haemolysis, on an individual case-to-case basis. Treatment with eculizumab may be continued for up to at least 6 weeks postpartum.[a]	2
Statement 7.2	In pregnant women with PNH with associated risk factors for thrombosis and no known contraindication to anticoagulants, prophylactic or therapeutic anticoagulation (with low molecular weight heparin) may be initiated and continued for up to at least 6 weeks postpartum.[b]	2
Statement 7.3	The frequency of monitoring may be increased in PNH patients who are pregnant after detailed assessment on a case-to-case basis, regardless of the ongoing treatment.	5

AA: aplastic anaemia; MDS: myelodysplastic syndrome; PNH: paroxysmal nocturnal haemoglobinuria
[a] Further continuation of eculizumab may be at the clinician's discretion on a case-to-case basis
[b] Duration of thromboprophylaxis postpartum may be extended at the clinician's discretion on a case-to-case basis

is administered subcutaneously every 4 weeks and can be self-administered. It may be a treatment option for patients with rare C5 mutations not benefitting from eculizumab or ravulizumab treatment.[72] Three large phase III studies in patients previously treated with eculizumab (COMMODORE 1; ClinicalTrials.gov identifier: NCT04432584) and patients naive to standard complement inhibitors (COMMODORE 2; NCT04434092 and COMMODORE 3; NCT04654468) are ongoing to confirm its efficacy and safety.[22,73] Preliminary results showed thatcrovalimab is non-inferior to eculizumab for the control of haemolysis and transfusion avoidance, with a well-tolerated safety profile.

Building on the success of pegcetacoplan, a variety of proximal inhibitors have emerged. Notably, several of these inhibitors have reached advanced stages of development for treating PNH. Recently, oral iptacopan (inhibitor of factor B) was approved as a breakthrough therapy for PNH based on the positive phase II data.[72] In phase II trials with iptacopan, 10 clinical BTH events were reported; all were mild or moderate except 1 severe event.[74] Other oral C3 inhibitors under development are danicopan, BCX9930 and vemircopan.[9] The rationale behind combining terminal and proximal inhibitors is to enhance control and effectively prevent both intravascular and extravascular haemolyses. The preliminary results of the ALPHA trial, a phase III double-blind RCT, showed that add-on danicopan versus placebo in patients receiving eculizumab or ravulizumab significantly improved haematological responses by addressing EVH while maintaining control of IVH.[75] These emerging therapies will expand the PNH therapeutic armamentarium and help improve the quality of life of patients with PNH.

Access to emerging treatments for PNH in Singapore

Currently, eculizumab is the only complement inhibitor approved by the Health Sciences Authority for the treatment of patients with PNH in Singapore. However, limited access to treatment poses a significant barrier to optimising the management of PNH. In Singapore, the Rare Disease Fund (RDF), established in 2019, utilises a multi-party funding model (with community-government donation ratio of 1:3) to support drug treatment for rare disease patients. RDF has a limited budget to meet the huge unmet need of innovative rare disease treatments. The process of inclusion of a rare disease drug into the RDF involves evaluation of proposals based on predefined criteria by the Rare Disease Expert Group and Rare Disease Fund Committee, with technical support from the Agency for Care Effectiveness (ACE).

The second option to improve accessibility of eculizumab for patients with PNH is to enlist in the cancer drug list (CDL). Until September 2022, all cancer drug treatments were fully covered by MediShield Life (MSL) up to SGD3000/month with additional support from Integrated Shield Plans (IP) and the Medication Assistance Fund (MAF). From September 1, 2022, only treatments listed on the CDL receive MSL coverage. Furthermore, IP insurers have aligned outpatient cancer coverage plans according to CDL from April 2023, with non-CDL drugs being non-claimable under IPs. In addition to these challenges, ACE/healthcare practitioner-led submission of application for enlisting drugs into the CDL usually takes 12–18 months, while company-led submission requires about 12 months.

The coverage of eculizumab treatment cost under the RDF and/or enlisting of eculizumab into the CDL will help improve its access and allow early intervention and improved treatment outcomes for patients with PNH in Singapore.

CONCLUSION

The 16 statements on the screening, diagnosis, treatment and monitoring of PNH presented in this consensus paper have been drafted based on a comprehensive review of the literature and real-world clinical practice sharing by PNH experts in Singapore. Based on the best available evidence, we have outlined the current guidance on the diagnosis and management of PNH in Singapore. Nevertheless, it may be warranted to refine the recommendations in the future as more evidence evolves.

Supplementary Materials

Fig. S1. *Literature search and article screening strategy.*

Fig. S2. *Multidisciplinary management of classic PNH (Statements 5.1–5.8).*

Table S1. *Oxford Centre for Evidence-Based Medicine 2011 levels of evidence.*

Table S2. *Bibliography for additional reading.*

Table S3. *Central themes for the consensus recommendations and summary of agreement ratings from the 2 rounds of Delphi voting.*

Conflict of interest

YTG received consultancy fees for participating in scientific advisory board meetings from AbbVie, Amgen, Antengene Corp, Astellas, AstraZeneca,

DKSH, GlaxoSmithKline, Janssen, Novartis, Pfizer, Recordati, Roche and Sanofi.

ESY, CWT, DT, YSL, LLC, ZYL and HT declare no conflict of interest.

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