Case Report

Aplastic anemia with paroxysmal nocturnal hemoglobinuria clones at diagnosis: Overlap, challenges, and therapeutic dilemmas

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ABSTRACT

Aplastic anemia (AA) is a rare, potentially life-threatening bone marrow failure syndrome characterized by pancytopenia and hypocellular marrow. Clonal evolution, particularly the coexistence of paroxysmal nocturnal hemoglobinuria (PNH) clones, has important diagnostic and prognostic implications. We report the case of a 25-year-old male who presented with recurrent mucosal bleeding, progressive fatigue, exertional dyspnea, weight loss, and fever. Initial evaluation revealed severe pancytopenia with reticulocytopenia and markedly hypocellular bone marrow showing aplasia. Flow cytometry demonstrated PNH clones within red cells (0.03%), granulocytes (8.35%), and monocytes (6.45%). The absence of hemolysis, elevated lactate dehydrogenase, or thrombosis supported AA as the primary disease. The patient was managed with transfusion support, antifibrinolytics, and was listed for hematopoietic stem cell transplantation. The coexistence of PNH clones in AA complicates diagnosis due to overlapping clinical features but also carries prognostic implications. Small PNH clones are increasingly detected with high-sensitivity fluorescent labeled aerolysin-based flow cytometry and may predict a favorable response to immunosuppressive therapy. However, clonal expansion over time can lead to classical PNH with hemolysis and thrombosis, underscoring the need for serial monitoring. This case highlights the importance of routine PNH clone screening in AA patients at diagnosis. Even small, asymptomatic clones can influence prognosis and necessitate long-term surveillance.

Key words: Aplastic anemia, Bone marrow failure, Flow cytometry, Hematopoietic stem cell transplantation, Paroxysmal nocturnal hemoglobinuria, Paroxysmal nocturnal hemoglobinuria clones

Aplastic anemia (AA) is a rare, life-threatening bone marrow failure syndrome characterized by peripheral pancytopenia and hypocellular marrow, often presenting as a diagnosis of exclusion. The incidence is estimated at 2/million in Western populations and 4–6/million in Asian countries, including India [1,2]. Although the majority of cases are idiopathic, secondary etiologies include exposure to cytotoxic drugs, chemicals, viral infections, and autoimmune mechanisms [1]. An important phenomenon in AA is clonal evolution, where patients may develop related hematological disorders such as paroxysmal nocturnal hemoglobinuria (PNH), myelodysplastic syndromes (MDS), or acute myeloid leukemia [3]. The association of AA with PNH clones is particularly well described: up to 50% of AA patients may harbor small PNH clones during the disease course, and some later evolve into clinical PNH [4-6]. These small, often asymptomatic clones are increasingly identified with the use of high-sensitivity fluorescent labeled aerolysin-based flow cytometry. Importantly, their detection has both diagnostic and prognostic implications, as they may predict response to immunosuppression therapy while simultaneously posing long-term risk of clonal expansion.

Access this article online	
Received - 17 September 2025 Initial Review - 30 September 2025 Accepted - 24 October 2025	Quick Response code
DOI: ***	

CASE REPORT

A 25-year-old male presented to us with complaints of nasal, per rectal, and gum bleeding for 1 month, which was intermittent. He reported spontaneous episodes of epistaxis for 2–3 days, which settled by itself. He also reported melena for the past 20 days. While brushing, he had intermittent gum bleeds despite good oral hygiene. These episodes of bleeding had increased in frequency over the week. He also had weight loss for 1 month, breathlessness on exertion, easy fatigability in the past

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7 days, fever, and headache for 5 days. There were no complaints of hematemesis, abdominal pain, nausea, vomiting, or abdominal distention. The patient was referred from a private hospital where he was treated with 4 pints of transfusion of platelet concentrate, and pancytopenia was confirmed by investigation, hemoglobin: 4.5, white blood cell: 1100, platelets: 10,000. The patient had a history of fever, which was presumed to be enteric, 1 month back, and no similar complaints in the past. No significant family history was found.

General and systemic examinations were normal except for the presence of pallor and a few petechiae. He had no episodes of hematuria or any evidence of deep vein thrombosis.

On evaluation, the patient was pancytopenic with investigations on admission and on discharge, as shown in Table 1. Bone marrow aspiration revealed aplastic marrow with markedly suppressed hematopoiesis, hypocellular, decreased erythroid and myeloid precursors, and absent megakaryocytes. Anti-nuclear antibody was positive by immunofluorescence. Antinuclear antibody (ANA) profiling was negative. Flow cytometric analysis demonstrated PNH clones (RBC 0.03%, granulocytes 8.35%, monocytes 6.45%), consistent with AA with subclinical PNH clones, without clinical hemolysis.

The biochemical evaluation revealed mildly elevated lactate dehydrogenase (LDH) (320 U/L), while renal function tests (urea 27 mg/dL, creatinine 1.17 mg/dL), and liver function parameters, including bilirubin, transaminases, and alkaline phosphatase, were within normal ranges. Serum proteins, albumin, and globulin were also normal. Interestingly, serum ferritin (1260 ng/mL) and iron levels (215 µg/dL) were elevated, with markedly high transferrin saturation (93%) and reduced total iron-binding capacity (232 µg/dL), suggesting iron overload, likely secondary to repeated transfusions. Vitamin B12 was elevated (>2000 pg/mL). Thyroid profile was within normal limits. Infectious screening, including tests for human immunodeficiency virus (HIV), hepatitis B, hepatitis C, malaria, typhoid, dengue, and chikungunya, parvovirus B19, was negative. Stool examination and blood cultures were unremarkable. Overall, these findings excluded secondary causes of cytopenia and supported the diagnosis of AA with PNH clones.

Table 1: Investigations of the patient on admission and on discharge

Parameter	On admission	On discharge	Reference range
Hemoglobin (g/dL)	4.4	7.2	13–17
RBC (million/mm^3)	1.87	2.48	4.5–5.5
WBC (cells/mm^3)	2000	1600	4000–10000
Platelets (cells/mm^3)	20000	12000	150000–400000
Reticulocyte count (%)	0.5	0.5	0.5–2.5
Peripheral smear	Mild anisopoikilocytosis, - hypochromic RBC, many elliptocytes, leukopenia, platelets reduced on smear		
ESR	98	37	0–10
PT (sec)	15.1	12.7	11–14
INR	1.01	1.12	-
aPTT	22.7	24.5	28–36
Sodium (mEq/L)	143	140	13–145
Potassium (mEq/L)	3.2	4.2	3.5–5.1

RBC: Red blood cells, WBC: White blood cells, ESR: Erythrocyte sedimentation rate, PT/INR: (Prothrombin Time/International Normalized Ratio, aPTT: Activated partial thromboplastin time

Supportive treatment in the form of blood transfusion, tablet tranexamic acid was given, and the patient currently awaits a donor for hematopoietic stem cell transplant (HSCT). Given severely hypocellular marrow, spontaneous hematologic recovery is unlikely. The patient is on follow-up twice weekly for monitoring of cell counts along with platelets. Counseling regarding hygiene, prevention of infection, and care to prevent sepsis was explained and is being reinforced on follow-up. The patient is currently transfusion dependent and on oral iron chelators to prevent secondary hemochromatosis.

DISCUSSION

This patient's presentation reinforces the complex interplay between AA and PNH clones and highlights important considerations in diagnosis, prognostication, and management.

Overlap in clinical features and pathophysiology: AA and PNH share non-specific features such as fatigue, mucocutaneous bleeding, and dyspnea, making the initial distinction difficult [7]. PNH is caused by somatic mutations in the *PIGA* gene, leading to deficient expression of glycosylphosphatidylinositol (GPI)–anchored proteins (notably CD55 and CD59), rendering affected cells susceptible to complement-mediated lysis. In the setting of immune-mediated bone marrow failure, such as AA, a prevailing hypothesis proposes that PNH (GPI-deficient) hematopoietic stem and progenitor cells may evade autoimmune T-cell attack that preferentially targets normal (GPI-expressing) progenitors. Thus, in marrow suppression, these clones may have a relative selective advantage and expand [5,8].

In practice, most PNH clones detected in AA are small and subclinical. Approximately two-thirds of PNH clones occur in patients whose clinical phenotype is bone marrow failure rather than classical PNH with overt hemolysis or thrombosis.

Prevalence of PNH Clones in AA and natural history: Multiple studies show that 30–60% of newly diagnosed AA cases harbor a PNH clone detectable by high-sensitivity flow cytometry. For example, in a large retrospective series of 207 severe AA patients, 40% had a detectable clone pre-treatment with a median granulocyte clone size of 9.7% (IQR 3.5–29%) [9]. After immunosuppressive therapy (IST), in many patients,

the clone size remains stable or even decreases; in a minority, expansion may occur. In that same cohort, about 30 patients (of 83 with a baseline clone) showed an increase in clone size post-IST, but only 7 had clinical PNH requiring therapy, all of whom had elevated LDH and clone sizes >50% [9].

The dynamic evolution of PNH clones in AA has been more recently modeled. In a study of evolutionary patterns, AA cases that progressed to clinical hemolysis after IST had significantly larger clones (granulocytes: 12.3% vs. 2.6%; erythrocytes: 5.7%) compared to those that did not convert, suggesting that baseline clone size and post-therapy kinetics may predict progression risk [8].

Prognostic role of PNH clones

The presence of PNH clones at diagnosis in AA has been associated in many studies with a more favorable response to IST. In a well-known prospective study of 125 AA patients treated with IST, PNH+ patients (59%) had better 6-month response rates (68% vs. 45%; p=0.0164), and higher rates of complete remission (42% vs. 16%) compared to PNH– patients. In multivariate analysis, PNH clone presence (odds ratio [OR] 2.56) and higher baseline reticulocyte count were independent predictors of response [8]. A more recent meta-analysis pooling 15 studies (n=1,349) showed that pre-treatment PNH clone positivity predicted better hematologic responses at 6- and 12-month intervals (pooled ORs ~1.49 for 6 months; ~3.10 for 12 months) and overall response (pooled OR ~1.69). Interestingly, they also found that PNH+ patients were more likely to progress into AA/PNH syndrome after IST (OR ~2.78) [10]. Another meta-analysis (Karger) similarly concluded that PNH clone presence has an evidence-based role in predicting better responses to immunosuppression [11]. However, not all recent data are consistent. A study (416 patients) that included patients treated with IST±eltrombopag observed that platelet count and presence of a PNH clone did not significantly correlate with response; rather, higher pre-treatment reticulocyte and neutrophil counts (and lower thrombopoietin) were better predictors [12].

Thus, although the presence of clones is broadly prognostically favorable in many cohorts, the relationship is not absolute and may interact with other baseline hematologic parameters and therapy regimens (e.g., addition of thrombopoietic agents).

Therapeutic implications and monitoring strategy

In AA patients with subclinical PNH clones, first-line therapy remains guided by AA severity: HSCT (in young patients with a matched donor) or IST. The presence of small clones does not change the initiation of therapy, but may influence monitoring intensity and donor search urgency. In some cases where large PNH clones coexist or expand and manifest hemolysis or thrombosis, complement blockade (e.g., eculizumab) may be used, sometimes bridging to transplant. For example, eculizumab may be given before HSCT to mitigate hemolytic and thrombotic complications [13]. A notable case series from the UK described 25 patients with concurrent AA/PNH treated with eculizumab and IST or HSCT, with varying outcomes [14]. Given the possibility of clonal expansion or transformation, serial monitoring is essential. Regular flow cytometry (e.g., every 6–12 months), surveillance for hemolysis markers, and vigilance for cytogenetic or somatic mutation emergence are recommended [5].

Application to our case and limitations

In our patient, although the RBC clone was very low (0.03%), the granulocyte and monocyte clones (8–6%) are within the range seen in marrow failure–associated PNH clones. Given the absence of hemolysis or thrombosis, the clone is subclinical. However, detection is prognostically valuable and mandates closer follow-up. We acknowledge limitations: (1) inability to perform serial clone dynamics for longer duration; (2) absence of detailed immune profiling or somatic mutation assessment; (3) lack of larger regional case comparisons in India. Future reporting of Indian cohorts could further delineate clone behavior in different genetic/ethnic settings.

In addition, in patients presenting with pancytopenia and hypocellular marrow, several differential diagnoses must be carefully considered before confirming AA with coexisting PNH clones: MDS/acute.

Leukemias

Excluded in this case as there was no morphological dysplasia on marrow examination, no cytogenetic abnormalities, and the absence of blasts. Nutritional deficiencies such as Vitamin B12, folate, or iron. Secondary causes of marrow suppression, particularly infections (HIV, hepatitis B/C, parvovirus B19, dengue, chikungunya, enteric fever) and toxin/drug exposures, are recognized etiologies. Autoimmune cytopenia: Systemic lupus erythematosus may present with pancytopenia, but autoantibody profiling (ANA positive but ANA panel negative) and marrow aplasia were more consistent with AA.

CONCLUSION

In summary, this case illustrates the diagnostic complexity of severe AA with coexisting small PNH clones, underscoring the importance of a thorough differential diagnosis to exclude mimicking conditions such as MDS, acute leukemia, nutritional deficiencies, and autoimmune cytopenia. While the detected clones were subclinical, their presence carries significant prognostic implications, particularly regarding response

to IST and the risk of clonal evolution. Routine high-sensitivity flow cytometry at diagnosis, careful long-term monitoring, and timely consideration of hematopoietic stem cell transplantation remain critical in optimizing outcomes for such patients.

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Funding: Nil; Conflicts of interest: Nil.

How to cite this article: Sirajwala AA, Raval RD, Patel RY, Bodar DA. Aplastic anemia with paroxysmal nocturnal hemoglobinuria clones at diagnosis: Overlap, challenges, and therapeutic dilemmas. Indian J Case Reports. 2025; October 31 [Epub ahead of print].