**INTRODUCTION**

Paroxysmal nocturnal hemoglobinuria (PNH) is a life-threatening acquired hematopoietic stem cell disorder characterized by deficiency of the glycosylphosphatidylinositol (GPI) anchor enzyme that provides protection against C5 and C9.

This deficiency results in unregulated, complement-mediated, chronic hemolysis (e.g. aplastic anemia, myelodysplastic syndrome), leading to serious morbidities, including thrombocytopenia, chronic kidney disease, and increased mortality.

The International Clinical Consultancy Society (ICCS) recommends that patients with clinical indications of bone marrow failure, hemolytic/hemoglobinuria, unexplained cytopenias, or unexplained thrombosis be tested for PNH.

- High-sensitivity flow cytometry is the recommended method for diagnosing PNH.
- Both white blood cell (WBC) granulocytes and monocytes and red blood cell (RBC) erythrocytes testing are recommended for initial diagnosis.
- WBC testing in granulocytes and monocytes populations provides the most accurate estimate of the true PNH clone size.

PNH testing in RBC and WBC populations is typically smaller than PNH WBC clones because of hemolysis and/or transfusion, and RBC testing alone should not be performed.

The incidence of PNH clones in these patient groups has been reported to date.

**OBJECTIVE**

To analyze the distribution of PNH clone sizes by high-sensitivity flow cytometry among high-risk patient groups based on international Classification of Diagnoses, ICD revision (ICD-9) diagnostic code and to evaluate the impact of hemolysis on PNH clone size.

**METHODS**

- The distribution of PNH clone population sizes were compared with ICD-9 diagnostic codes (Table 1) for 7699 patients recommended for testing by the ICCS and the PNH Internet Group, who sent samples to Dahl-Chase Diagnostic Services, Bangor, Maine.
- High-sensitivity flow cytometry analysis: peripheral blood specimens were analyzed on Beckman Coulter FC 500 flow cytometers with a panel to detect a small population of 0.01%.

- A combination of CD24-PE, CD59-PE, and FLAER-ALEXA antibodies was used for RBC and WBC analysis, including fluorescent-labeled reactive reagents (FLER), which is a unique protein that binds tightly and specifically to mammalian GPI anchors.

**RESULTS**

- ICD-9 diagnostic groups were further analyzed for granulocytes and erythrocytes.

- The majority of patients (70.9%) were found to be PNH positive by flow cytometry analysis and were recommended for therapy. This was followed by patients in the aplastic anemia category (35.1%) and multiple myeloma (22%).

**DISCUSSION**

- In patients with both aplastic anemia and hemolytic ICD-9 diagnoses (n=236), 36.8% had PNH population proportion sizes >20% (Figure 3B).
- PNH clone population proportion sizes >20% in 5 patients (33.3%), 1–20% in 4 patients (26.7%), and ≤1% in 5 patients (33.3%).

- In patients with both myeloproliferative syndrome or cytopenia and hemolytic ICD-9 diagnoses (n=20), 65.5% had PNH population proportion sizes >20% (Figure 3).

**CONCLUSIONS**

- This study confirms the need to continue actively testing high-risk patient populations, including those with aplastic anemia, myelodysplastic syndrome, unexplained cytopenias, unexplained thrombosis, and hemolytic, for PNH based on the ICCS’s recommendations to ensure accurate diagnosis and appropriate therapy.

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**REFERENCES**


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