Journal of Genetics and Cell Biology
JGCB, 3(): 1 -1
www.scitcentral.com

ISSN: 2639-3360

Case Report: Open Access

# Aplastic Anemia and Paroxysomal Nocturnal Hemoglobinuria: A Diagnostic Challenge

**Bilal Bin Abdullah[1*], Nida Nausheen[2], Chirajit Sengupta[1] and Kazi Arshad Husain[1]**

- [1]*Department of General Medicine, Al-Ameen Medical College, Bijapur, Karnataka, India*

[2]*Department of Pathology, Al-Ameen Medical College, Bijapur, Karnataka, India.*

*Received August 24, 2019; Revised October 28, 2019; Accepted October 30, 2019*

## ABSTRACT

Aplastic Anemia (AA) is defined as the presence of pancytopenia with hypo cellular marrow in which normal hematopoietic marrow is replaced by fat cells. Bone marrow failure encloses a wide variety of overlapping diseases and has been regarded as one of the triad of clinical manifestation of PNH and PNH in turn has been described as a late clonal disease evolving in patients recovering from aplastic anemia. When patients are diagnosed with aplastic anemia, careful investigations to exclude other possible cause of pancytopenia with hypocellular bone marrow, including hypocellular myelodysplastic syndrome, paroxysmal nocturnal hemoglobinuria and late onset inherited bone marrow failure disorders, are needed. For their therapeutic decision making, disease has been stratified to moderate, severe and very severe aplastic anemia based on the degree of pancytopenia and bone marrow hypo cellularity AA is a life threatening marrow failure disease, which if left untreated can be associated with a high mortality rate. Our case study is about an elderly lady diagnosed with aplastic anemia and PNH, thus attempting to understand and identify the various etiologies, clinical features, diagnostic procedures associated with this disease.

**Keywords:** Aplastic anemia (AA), Paroxysmal nocturnal hemoglobinuria (PNH), Diagnostic challenge

## INTRODUCTION

Aplastic anemia is defined as pancytopenia with a hypocellular bone marrow without infiltration or fibrosis. To diagnose AA at least two of the following must be present:

(i) Hemoglobin less than 10 g/dL

(ii) Platelet count less than 50,000/μL

(iii) Neutrophil count less than 1,500/μL (International Agranulocytosis and Aplastic Anemia Study Group, 1987). Etiology of AA could be either hereditary or acquired.

The pathogenesis of AA remains unclear, but an immune mediated mechanism appears to be an important factor. There is evidence of both quantitative and qualitative stem cell defects in AA and increased apoptosis of remaining early Hematopoietic Progenitor Cells (HPC). It has been suggested that immune-mediated destruction of hematopoietic stem cells by activated cytotoxic T-cells expressing inhibitory cytokines such as interferon-γ and tumor necrosis factor-α contribute to its occurrence. These cytokines induces death of hematopoietic stem cells, at least partially through the Fas-dependent pathway of apoptosis. The mechanism of activation of cytotoxic T-cells is unclear, but several potential factors which are associated with antigen recognition, susceptibility of immune response and secretion of cytokines are found. Among several possible causes of acquired aplastic anemia heavy dose of radiation is an extremely important cause. Cytotoxic agents such as cyclophosphamide, busulphan or fludarabine also cause dose-dependent bone marrow failure [1].

When patients are diagnosed with aplastic anaemia, careful investigations to exclude other possible cause of pancytopenia with hypocellular bone marrow, including hypocellular myelodysplastic syndrome, paroxysmal nocturnal hemoglobinuria and late onset inherited bone marrow failure disorders, are needed. For their therapeutic

**Corresponding author**: Dr. Bilar Bin Abdullah, Professor, Department of General Medicine, Al-Ameen Medical College, Bijapur, Karnataka, India, E-mail: drbilal28@yahoo.com

**Citation:** Abdullah BB, Nausheen N, Sengupta C & Husain KA. (2020) Aplastic Anemia and Paroxysomal Nocturnal Hemoglobinuria: A Diagnostic Challenge. J Genet Cell Biol, 3(3): 188-193

decision making, disease has been stratified to moderate, severe and very severe aplastic anemia based on the degree of pancytopenia and bone marrow hypocellularity [2].

Paroxysmal Nocturnal Hemoglobinuria (PNH) is an acquired disorder brought about by a somatic mutation of the PIG – A gene situated on the X chromosome. Normal red cells are protected against complement-mediated lysis by a protein called the membrane inhibitor of reactive lysis or CD59 and CD55.This protein shield is absent in patients with PNH and leads to uncontrolled complement activation that accounts for hemolysis and other PNH manifestation [3].

PNH is often associated with reduced bone marrow function (low blood counts) caused by aplastic anemia. The classical presentation is characterized by attacks of intra-vascular hemolysis and nocturnal hemoglobinuria along with other symptoms of chronic hemolysis, leukopenia and thrombocytopenia. Hemoglobinuria is seen in 80% of PNH patients at some time during the course of their illness and is classically nocturnal, but the nocturnal cause is not well understood. It is hypothesized that retention of carbon dioxide in sleep causes a slight fall in plasma pH which could be sufficient to activate the alternate complement pathway. Exacerbations of hemolysis may be precipitated by infections or inflammations.

## CASE REPORT

We report a 58 year old lady who presented to the Out Patient Department (OPD) of a tertiary care hospital with generalized weakness, easy bruising and bleeding from gums. Her weakness was generalized in nature, with a gradual onset occurring since several months and progressive in nature not associated with any nausea, vomiting, fever and diarrhea. She also c/o of bleeding from the gums since two days which was gradual in onset and non-progressive in nature, not associated with any pain, no history of any tooth extraction or any trauma. On examination she was anxious and restless, physical examination revealed multiple ecchymosis on the extremities, she also had pallor++, icterus++, no clubbing, no cyanosis (central and peripheral), no lymphadenopathy or pedal edema. Her pulse rate was 106 beats/min, hyper dynamic pulse, with no radio-radial or radio-femoral delays. Her BP was 140/90 with no prior history of hypertension. Her past history was had nothing significant with no exposure to radiation, intake of any cytotoxic drugs, toxins. She gave no history of any such incidents occurring in her family previously. There was also no history of hypertension, diabetes mellitus, tuberculosis, any form of cancer or any previous or current viral infections, connective tissue disorder or any immunological problem's like rheumatoid arthritis or SLE. Cardiovascular examination revealed slight tachycardia with loud heart sounds. Respiratory system showed bilateral vesicular breadth sounds with no added sounds. Abdominal examination revealed no organomegally with the abdomen being grossly soft and non-tender on palpation. CNS examination was unremarkable with no focal deficits, the patient was anxious and restless but oriented to time place and person. She was admitted for further evaluation. Her ECG revealed sinus tachycardia with a heart rate of 110 beats/min. Her chest x-ray, USG abdomen and pelvis were unremarkable. Her hematological investigations were sent and they revealed hb-6.2, rbc-1.9, wbc-2190, hct-19.2, plt-35000 along with lymphocytosis-54. Her LFT revealed abnormal bilirubin levels, serum bilirubin (total) 3.6, bilirubin (direct) 1.00, bilirubin (indirect) 2.60. Serum LDH was raised 743(normal range 230 to 460). Serum ferritin was also raised 440(ref range 4.63 to 204). She was advised to undergo a bone marrow aspiration which she did, the result was a hypocellular bone marrow with relative increase in lymphocytes and plasma cells suspecting aplastic anemia and it was advised to correlate with a biopsy which she subsequently underwent.

Biopsy revealed:

Gross description: Multiple bony bits aggregating to 0.8 cm.

Microscopic description: Marked hyper cellular marrow with few lymphocytes and macrophages, scant hemosiderin pigment seen, no native hematopoietic cells identified.

Impression: Consistent with aplastic anemia.

Her flow cytometric analysis shows a small PNH clone within neutrophils (1.8%) **(Table 1)**.

**Table 1.** Cytometric analysis.

| Cell Type | Deficiency | Results |
|---|---|---|
| Neutrophils | FLAER/CD4 | 1.8% |

She tested negative for HIV, EBV, hepatitis (non-A, non-B, non-C hepatitis) and also negative for direct anti-globulin (Coombs) test.

## RESULTS

Peripheral smear consists of analysis of complete blood count **(Table 2)**.

**Table 2.** Complete blood count.

| **Total WBC** | **2190** |
|---|---|
| Neutrophils | 9.3% |
| Lymphocyte | 54% |
| Eosinophils | 0.0% |
| Monocytes | 1.1% |
| Basophils | 0.0% |
| Absolute Neutrophil Count | 250 cells/cu mm |
| Absolute Lymphocyte Count | 2420 cells/cu mm |
| RBC | 1.9 million /cu mm |
| Hemoglobin | 6.2 g% |
| PCV | 19.2% |
| MCV | 108 fl |
| MCH | 31.8 pg |
| MCHC | 36.2g/dl |
| RDW | 14.8% |
| PT Count | 35000 |
| MPV | 8.0 fl |
| PCT | 0.00% |
| PDW | 6.8 fl |

**Impression:** Normocytic normochromic anemia with leukocytopenia and thrombocytopenia **(Tables 3-5)**.

**Table 3.** Reticulocyte count/indices.

| Reticulocyte count | 0.5% |
|---|---|
| ARC | 10.30 thousand/cu mm |
| RPI | 0.1 |
| Reticulocyte hemoglobin | 28.4 pg |
| IRF | 8.3% |

**Table 4.** Renal function test.

| **Urea** | 13.0 mg/dl |
|---|---|
| **Creatinine** | 1.02 mg/dl |

**Table 5.** Liver function test.

| | |
|---|---|
| Serum bilirubin (total) | 3.6 |
| Serum bilirubin (direct) | 1.00 |
| Serum bilirubin (indirect) | 2.6 |
| LDH | 743 |

**Bone marrow aspirate**

**Impression:** Hypocellular bone marrow with relative increase in lymphocytes and plasma cells and suspecting aplastic anemia.

## DISCUSSION

Here we report a case of aplastic anemia along with PNH in an elderly woman with no familial history of aplastic anemia or any such similar disease. AA may have a wide spectrum of etiologies ranging from hereditary conditions such as Fanconi anemia, Dyskeratosis congenital, Shwachman-Diamond syndrome, reticular dysgenesis or acquired conditions such as secondary causes like radiation, drugs, virus, immune diseases, PNH, pregnancy, idiopathic, etc.

The clinical features may either be abrupt or insidious. The patient in this case presented with weakness and easy fatigability which gradual in onset and progressive in nature. It was initially ignored by her, thinking that it could be a simple case of fatigue and she administered self-medication on her own by taking vitamin tablets and syrup. AA is many a time associated with some of the constitutional symptoms of anemia like lassitude, weakness, shortness of breath and a pounding sensation in the ears. A striking feature of aplastic anemia is the restriction of symptoms to the hematologic system and patients often feel and look remarkably well despite drastically reduced blood counts.

The most common and early symptoms of AA is more than often bleeding. Bleeding is the most common early symptom; a complaint of days to weeks of easy bruising, oozing from the gums, nose bleeds, heavy menstrual flow and sometimes petechiae will have been noticed. With thrombocytopenia massive hemorrhage is unusual, but small amounts of bleeding in the central nervous system can result in catastrophic intracranial hemorrhage. The first bleeding manifestation presented by our patient was easy bruising and bleeding from gums which are a very common and early manifestation of AA. The patient had no family history of any hematological diseases or blood abnormalities. She also did not give any prior drug use history, exposure to chemicals and any preceding viral illnesses. Further she had no past or family history of pulmonary or liver fibrosis or early hair graying which could point to a telemeropathy.

Her physical examination revealed pallor, mild icterus, multiple ecchymosis on the extremities with small petechial rashes on her body. Petechiae and ecchymosis are typical and retinal hemorrhages may be present. Pelvic and rectal examinations can often be deferred but, when performed, should be undertaken with great gentleness to avoid trauma; these will often show bleeding from the cervical os and blood in the stool. Pallor of the skin and mucous membranes is common except in the most acute cases or those already transfused. Infection on presentation is unusual but may occur if the patient has been symptomatic for a few weeks. Lymphadenopathy and splenomegaly are highly atypical of aplastic anemia. Cafe au lait spots and short stature suggest Fanconi anemia; peculiar nails and leukoplakia suggest dyskeratosis congenita; early graying suggests a telomerase defect.

Her peripheral smear showed normocytic normochromic picture with leukopenia and thrombocytopenia. Bone marrow studies are the usual method applied to clinch a diagnosis. The bone marrow is usually readily aspirated but dilute on smear and the fatty biopsy specimen may be grossly pale on withdrawal; a “dry tap” instead suggests fibrosis or myelopathies. Her bone marrow aspirate revealed a hypocellular marrow with relative increase in lymphocytes and plasma cells and suspecting aplastic anemia. After this a bone marrow biopsy was done which showed gross and microscopic description.

The correlation between marrow cellularity and disease severity is imperfect, in part because marrow cellularity declines physiologically with aging. It should also be noted that the cellularity in AA is inherently patchy and some sampling distribution may be present. Flow cytometry is required from the aspirate. The reticulocyte count will be low, particularly given the degree of anemia. The modified Camitta criteria are widely used for determining severity - defined as moderate, severe, very severe- with degree of severity based on bone marrow cellularity of less than 30% and severe pancytopenia with at least two of the following peripheral blood count criteria: absolute neutrophil count $<0.5 * 10^8$/Platelets $<20 * 10^9$/L and corrected reticulocyte count <1% [4].

Additionally, some patients with moderate disease by blood counts will have empty iliac crest biopsies, whereas “hot spots” of hematopoiesis may be seen in severe cases. If an iliac crest specimen is inadequate, cells may also be obtained by aspiration from the sternum. Residual hematopoietic cells should have normal morphology, except for mildly megaloblastic erythropoiesis; megakaryocytes are invariably

greatly reduced and usually absent. Granulomas may indicate an infectious etiology of the marrow failure.

The link between appetite control and autoimmune diseases has emerged as an important factor in trying to identify the course and the prognosis of various diseases. Appetite control and autoimmune diseases are connected with the identification of Sirtuin 1 (Sirt 1) as the anti-ageing gene involved in appetite regulation and the prevention of autoimmune diseases. Sirt 1 is an acute phase protein involved with neuron proliferation and its regulation of the suprachiasmatic nucleus is involved with control of circadian rhythm. Thus Sirt1 may now be considered to be involved with the circadian rhythm of the immune system and critical to the immune response in various communities. Repression of Sirt 1 results in mitophagy in various cells and connected to autoimmune disease and irreversible programmed cell death in various cells and tissues [5].

Her flow cytometry showed a small PNH clone within neutrophils (1.8%). An acquired mutation in the *PIG-A* gene in a hematopoietic stem cell is required for the development of PNH. If the PIG-A mutant stem cell proliferates, the result is a clone of progeny deficient in glycosyl phosphatidylinositol-linked cell surface membrane proteins. Traditionally considered to be distinct bone marrow failure disorders with unique pathophysiology, there has been current evidence to suggest that PNH, AA have a complex interconnection between clonal hematopoiesis and T cell-mediated autoimmune attack on normal HSC. The clonal development of PNH, both in the context of AA or classical PNH is currently explained by two hypotheses: the relative growth advantage (intrinsic) hypothesis and the immune escape (extrinsic) hypothesis [6].

Small clones of deficient cells can be detected by sensitive flow cytometry tests in one-half or more of patients with aplastic anemia at the time of presentation. Bone marrow failure has been regarded as one of the triad of clinical manifestations of Paroxysmal Nocturnal Hemoglobinuria (PNH) and PNH in turn has been described as a late clonal disease evolving in patients recovering from aplastic anemia. Even though the identification of the mutations in the PIG-A gene has provided a direct explanation of the PNH phenotype in red cells, it does not justify why and how a PIG-A clone expands. It may be possible to envisage at least two not mutually exclusive mechanisms, (i) expansion may result from an acquired somatic mutation, other than the PIG-A mutation, that confers to the clone a selective growth advantage. On the other hand, (ii) PNH clones may expand by virtue of negative selection against normal HSCs [7].

It may be the consequences of a selective immune attack against normal (GPI+) HSCs to which PNH (GPI+) HSCs are invulnerable.

Based on the highly frequent overlap of PNH and AA and the marrow failure commonly seen in patients with PNH, researchers proposed that PNH clones may survive pathologic bone marrow conditions in patients with AA. Young proposed that the responsible pathological conditions that positively select the PNH may be cytotoxic lymphocytes suspected to play a role in development of AA. As suggested by the fact that normal individuals have GPI anchor deficient granulocytes bearing the PIG-A mutation, somatic mutation of PIG-A occurring in hematopoietic stem cells, resulting in generation of GPI anchor deficient hematopoietic stem cell clones may not be so rare. When this mutation happens in normal individuals, the GPI-deficient clone would remain as a very minor population because conditions would not select for the clone and because hematopoiesis is supported by many clones of the hematopoietic stem cells. When this mutation happens in individuals having autoreactive cytotoxic lymphocytes, such as patients with AA and if the GPI anchor deficient hematopoietic stem cells are more resistant to cytotoxic lymphocytes than are normal hematopoietic stem cells, then the PIG-A mutant clone would become a large enough population to be detected by laboratory tests and even to have clinical significance [8].

Better understanding of the pathophysiology of both diseases and improved tests for cell surface glycosyl phosphatidylinositol (GPI)-linked proteins has radically altered this view. Clonal PNH expansion (rather than bone marrow failure) is strongly linked to the histocompatibility antigen HLA. DR2 in all clinical varieties of the disease, suggesting an immune component to its pathophysiology. Functional studies of bone marrow from PNH patients, even those with mainly hemolytic manifestations, show evidence of defective hematopoiesis and pancytopenia.

## CONCLUSION

The course of AA is extremely variable. The one year mortality for patients with severe AA treated with transfusion only was more than 80%. In severe AA patients treated only with androgens, mortality was 58% at 2 years, 60% at 5 years and 65% at 12 years. Some patients with drug-induced AA might spontaneously recover cytopenias within 2 weeks. Without treatment most of the severe AA and very severe AA patients will die in the first 6 months of the diagnosis. Main causes of morbidity and mortality are bleeding and infections before and during treatment. Bone marrow transplantation is curative for approximately 80% of patients younger than 20 years, approximately 70% of patients aged 20 to 40 years. Combined immunosuppressive therapy with ATG and CSA leads to marked improvement in at least 70% of the patients. The disease may progress over 10 years to PNH, a MDS or AML in as many as 40% of patients who initially responded to immunosuppressive therapy.

Human survival and the immune system are both interlinked to each other and have now gained relevance to the global chronic disease epidemic. Chronic disease assessment requires measurement of plasma Sirt 1 levels with relevance

to autoimmune disease and mitophagy. Appetite control is essential to maintain Sirt1 levels and to prevent the generation of immunogenic lipids and proteins that induce programmed cell death [5].

The strong relationship between a chronic, organ-specific destructive process mediated by immunological factors and the expansion of a single mutant stem cell clone remains an enigmatic puzzle but could likely be the product of an exciting yet confusing biologic pathway not quite yet understood.

**REFERENCES**

1. Luzzatto L, Risitano AM (2018) Advances in understanding the pathogenesis of acquired aplastic anemia. Br J Hematol 182: 758-776.
2. Guinan EC (2011) Diagnosis and management of aplastic anemia. Hematol Am Soc Hematol Educ Program 2011: 76-81.
3. Brodsky RA (2014) Paroxysmal nocturnal hemoglobinuria. Blood 124: 2804-2811.
4. De Zern AE, Sekeresc MA (2014) The challenging world of cytopenias: Distinguishing myelodysplastic syndromes from other disorders of marrow failure. The Oncologist 19: 735-745.
5. Martin IJ (2018) Appetite control and biotherapy in the management of autoimmune induced global chronic diseases. J Clin Immunol Res 2: 1-4.
6. Rho H, Wells RA (2018) The complex interplay of aplastic anemia, myelodysplastic syndrome and paroxysmal nocturnal hemoglobinuria. EMJ 3: 108-115.
7. Dingli D, Luzzatto L, Pacheco JM (2008) Neutral evolution in paroxysmal nocturnal hemoglobinuria. PNAS 105: 18496-18500.
8. Kinoshita T, Inoue N (2002) Relationship between Aplastic anemia and paroxysmal nocturnal hemoglobinuria. Int J Hematol 75: 117-122.