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**Original Research Article**

# Acquired idiopathic aplastic anemia: Study of 20 cases and review of literature

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## Abstract

Aplastic anemia is pancytopenia with hypocellular bone marrow having cellularity<25%, usually cellularity is <10%. In most cases, acquired aplastic anaemia behaves as a T- lymphocyte mediated immune disease, only those cases of pancytopenia with hypocellular bone marrow (Aplastic anaemia) were selected for study that were seronegative for HBSAG, ANTIHCV and HIV and chromosomal breakage study was also negative for Fanconi Anemia. Male female ratio was 1:1. In our study, 90% cases had bone marrow cellularity<10%, either came in severe or very severe category. 85% patients had ANC<500/µL, 20% had ANC<200µL and 30% presented with platelet count <10,000/µL .Thus usual presentation was pancytopenia with severe to very severe aplastic anemia having hemoglobin <6gm/dl, platelets <20,000/µL and absolute neutrophil count <500/µL. The patients presenting with pancytopenia, must be evaluated by peripheral blood smear and bone marrow studies. Bone marrow trephine biopsy is diagnostic. The immunosuppressive to Peripheral blood stem cell transplantation is potentially curative modality of treatment.

**Keywords:** Hypocellular bone marrow, Aplastic anemia, Pancytopenia, Immunosuppressive therapy.

## 1. Introduction

Aplastic anemia (AA) is pancytopenia with hypocellular bone marrow having cellularity<25%, usually cellularity is<10% .[1] In most cases, acquired aplastic anaemia behaves as a T- lymphocyte mediated immune disease. Cellular and molecular pathways have been mapped in some details for both effector (T-lymphocyte) and target hematopoietic stem and progenitor cells.[2,3] There is autoimmune bone marrow depression, leading to severe suppression of hematopoiesis, manifested by marked decrease in CD34+ progenitor cells.[5] It is morphologically characterized by empty spicules of marrow aspirate, fatty bone marrow core and occasional to no CD34+ in flowcytometry. It is broadly divided into acquired and inherited type (inherited bone marrow failure syndrome.[6] Acquired idiopathic type is common cause of aplastic anemia and very difficult to treat. Usual presentation is progressive weakness followed by abrupt fall in blood counts in otherwise normal paediatric to old persons. There is moderate to severe pancytopenia in various combination of anaemia, thrombocytopenia and leucopoenia with severe neutropenia, usually absolute neutrophil count (ANC) is <500/µL, and manifesting with shortness of breath, petechial rashes, mucosal bleeding and infections.[1, 4]

The annual incidence of acquired aplastic anemia is 2 cases per million person .In general; male and female are affected equally, but distribution is biphasic .Most cases of aplastic anemia are idiopathic; however radiation, chemicals, drugs, infections, and immunological diseases are possible causes in

secondary aplastic anemia.[7,8] Paroxysmal nocturnal haemoglobinuria (PNH) and myelodysplatic syndrome (MDS) clones are commonly associated.

## 2. Material and Methods

Retrospectively hematological and bone marrow data of 20 patients of hematology clinic were collected from January 2013 to December 2014. Only those cases of pancytopenia with hypocellular bone marrow (Aplastic anaemia) were selected for study that was seronegative for HBSAG, ANTIHCV and HIV and chromosomal breakage study was also negative for Fanconi Anemia. None had any congenital deformities. All cases had marrow cellularity<25%, predominantly had cellularity< 10%.Apart from ancillary investigations, complete blood count was done in 5-part CBC analyzer, peripheral smears of each case was made and bone marrow aspiration and biopsy was also done in each .Smears were also made for reticulocyte count and stained by Brilliant cresyl blue stain**.**

## 3. Result

In All cases initial presentation was pancytopenia (Table2, Fig.1A). Age ranged from 7 years to 65 years, average age was 29.2 year (table 2). Male female ratio was 1:1. In our study, 90% cases had bone marrow cellularity<10%, either came in severe or very severe category (Table 1& 2). 85% patients had ANC<500/µL, 20% had ANC<200µL and 30% presented with platelet count <10,000/µL. Thus usual presentation was pancytopenia with severe to very severe aplastic anemia having hemoglobin <6gm/dL, platelets <20,000/µL and absolute neutrophil count <500/µL. The red blood cell picture was normocytic normochromic to mildly macrocytic (MCV>100 to<110 fl). Corrected reticulocyte count was<1% in each and every case. Bone marrow aspiration smears showed only hypocellular fragments containing predominantly lymphocytes, plasma cells and few mast cells in the background of predominantly adipocytes (Fig. 1, B, C, D). Only occasional granulocytes, erythroblasts were found. In most cases (99%) megakaryocyte was not seen. Aspiration findings were confirmed on bone marrow trephine biopsy. In 25% cases, aspirates showed only blood. The histology of marrow biopsy showed cellularity <10% in 90% (Fig.2 A, B, C, D). In those cases where aspirates were not satisfactory, biopsy clinched the diagnosis. In 10% cases, biopsy showed occasional clusters of erythroblasts.

### 3.1 Classification of aplastic anemia based on severity of pancytopenia

A. **Severe aplastic anemia (SAA**)
Bone marrow cellularity<25%
Two of three peripheral blood criteria:
Absolute neutrophil count (ANC) <500/µL
Platelet count <20,000/µL
Corrected reticulocyte count<1%

**B. Very severe aplastic anemia (VSAA)**
Same as SAA with absolute neutrophil count (ANC) <200/µL

**C. Non severe (moderate ) aplastic anemia**
Bone marrow cellularity<25%
Peripheral blood cytopenias do not fulfil criteria for SAA

**Table 1**: **Hematological parameters of patients**

| Case No. | Sex | Age | TLC | ANC | HB | PLT | BM cellularity |
|---|---|---|---|---|---|---|---|
| 1 | F | 50 years | 2.0 | 300 | 3.6 | 20 | <10% |
| 2 | M | 10 years | 2.1 | 588 | 2.4 | 10 | <10% |
| 3 | M | 8 years | 2.5 | 450 | 4.5 | 15 | <10% |
| 4 | F | 65 years | 0.8 | 160 | 3.7 | 11 | <10% |
| 5 | F | 62 years | 1.5 | 260 | 5.0 | 25 | <10% |
| 6 | M | 18 years | 1.0 | 100 | 6.0 | 08 | <10% |
| 7 | M | 7 years | 1.4 | 280 | 4.6 | 09 | <10% |
| 8 | F | 15 years | 1.8 | 180 | 5.5 | 11 | <10% |
| 9 | F | 55 years | 2.5 | 450 | 5.8 | 18 | <10% |
| 10 | M | 27 years | 2.8 | 550 | 6.5 | 08 | <20% |
| 11 | F | 19 years | 2.5 | 510 | 4.4 | 06 | 15% |
| 12 | M | 30 years | 1.9 | 350 | 5.5 | 12 | <10% |
| 13 | F | 15 years | 0.9 | 110 | 2.5 | 10 | <10% |
| 14 | M | 14 years | 2.1 | 234 | 5.5 | 16 | <10% |
| 15 | M | 35 years | 2.6 | 510 | 6.5 | 25 | <18% |
| 16 | F | 46 years | 1.8 | 260 | 5.8 | 13 | <10% |
| 17 | F | 25 years | 0.7 | 150 | 2.3 | 7 | <10% |
| 18 | M | 13 years | 1.6 | 190 | 5.7 | 9 | <10% |
| 19 | M | 50 years | 2.2 | 200 | 4.5 | 10 | <10% |
| 20 | F | 20 years | 2.4 | 210 | 5.8 | 18 | 12% |

**F:** female, **M:** male, **TLC:** Total leukocyte count($x10^3$/µL), **ANC:** absolute neutrophil count (/µL), **HB:** haemoglobin (g/dL), **PLT:** platelet count($x10^3$/µL), **BM:** bone marrow

**Figure 1A: (40X) PBS showing pancytopenia; B (10X) bone marrow aspirate smear showing predominately fat cells & lymphocytes; C: (10X) BMA showing marrow fragments with mainly adipocytes; D: (40X) BMA smear showing only lymphocyte & Plasma Cells**

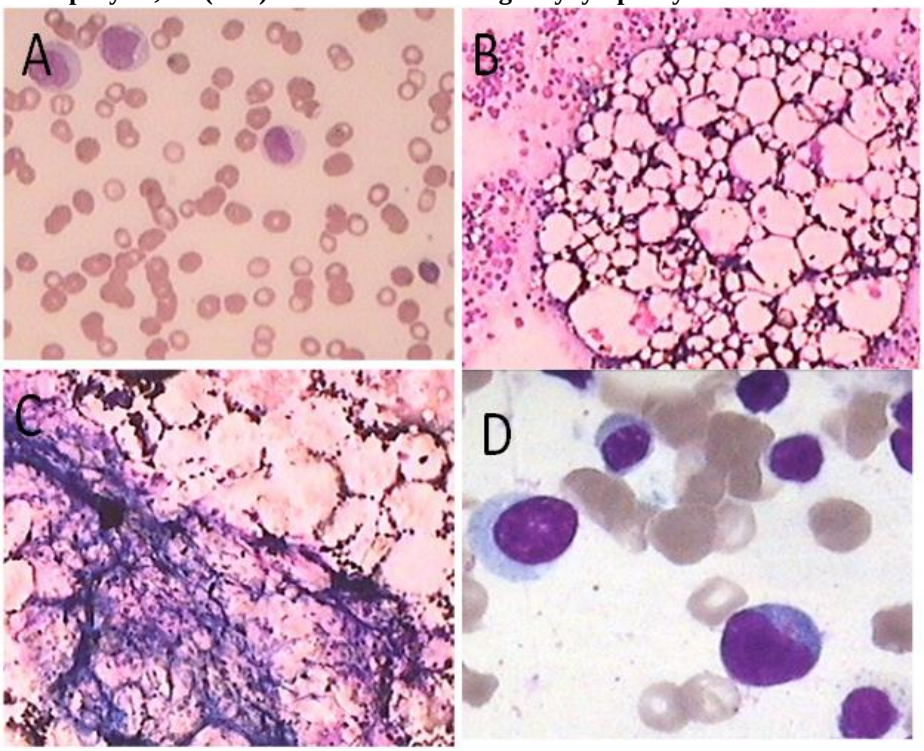
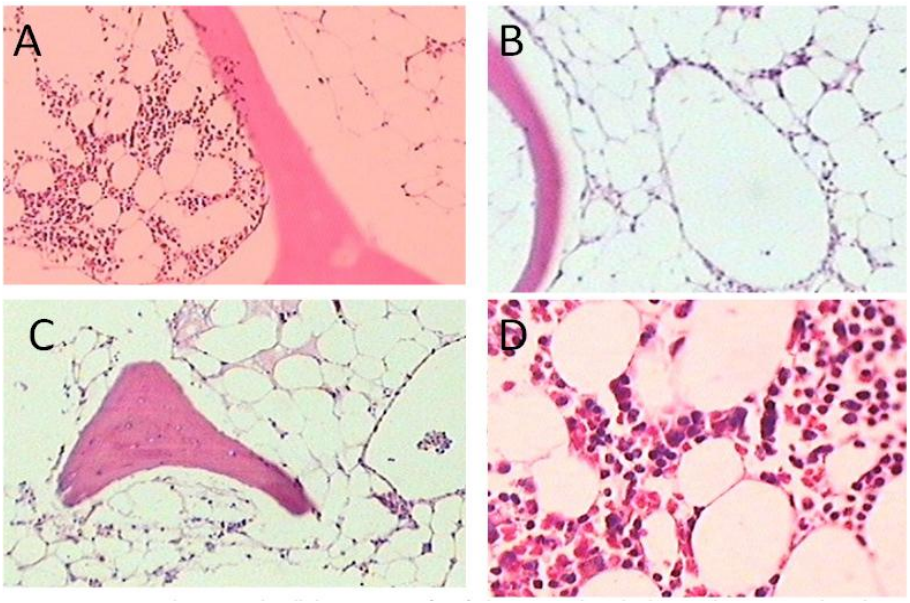



**Figure 2: Bone marrow biopsy with cellularity<10% A. (10X) showing trabecular bone, adipocytes & lymphocytes B. (10X) trabecular bone and prominent adipocytes C. (10X) trabecular bone, clusters of lymphocytes & predominantly adipocytes D. (40X) showing clusters of lymphocytes and scattered plasma cells**




## 4. Discussion

Aplastic anaemia (AA) is a rare but heterogeneous disorder. The annual incidence of acquired aplastic anemia is 2 cases per million persons in western world.[9] Thailand study determined 3.9 cases per million for the Bangkok metropolitan area and in Khonkaen, 5 cases per million.[10] Other Asian series have produced a range of 5 to 7 per million. Marked variations in the frequency of the disease, sometimes even within the same country or region, are suggestive of environmental factors influencing the occurrence of AA. The majority (70–80%) of these cases are categorised as idiopathic because their primary aetiology is unknown. [1,14] In a subset of cases, a drug or infection can be identified that precipitates the bone marrow failure, although it is not clear why only some individuals are susceptible. In approximately

15–20% of patients the disease is constitutional, where the disease is familial and presents with one or more other somatic abnormalities. [1-3]

The effector cells were identified by immunophenotyping as activated cytotoxic T cells (CD8+) expressing Th1 cytokines, especially γ-interferon and also tumour necrosis factor-α.[6]The cytokines secreted by CD8+ cells lead to apoptosis of CD34+ stem cells.[22] The recovery of hematopoietic progenitor cells CD8 cells and depletion of T-cells after a course of immunosuppressive therapy approves the theory of autoimmune cause of AA. T-cells containing intracellular interferon may now be measured directly in the circulation and oligoclonal expansion of CD8+ CD28– cells, defined by (1) flow cytometric analysis for T cell receptor (TCR) Vβ subfamilies; (2) spectratyping to detect skewing of CDR3 length; and (3) sequencing of the CDR3 region to establish a molecular clonotype.[6,32] kinectin, an autoantigen is present in 40% cases of AA.

The presence of associated paroxysmal nocturnal haemoglobinuria (PNH) and cytogenetic clone is common. Small PNH clones, in the absence of haemolysis, occur in up to 50% of patients of aplastic anaemia and abnormal cytogenetic clones occur in up to 12% of patients with aplastic anaemia in the absence of myelodysplatic syndrome (MDS).[28]

Moderate aplastic anaemia (MAA) in children is a rare, idiopathic condition of bone marrow insufficiency that can resolve spontaneously, persist for months or years, or progress to severe aplastic anemia (SAA). [4]

The aplastic anemia is major public health problem. Due to lack of bone marrow study in pancytopenic patients in rural areas and small cities of Bihar, they remained undiagnosed and patient succumbed to death within one month of presentation with pancytopenia. Bone marrow study is essential in pancytopenic patients to evaluate differential diagnoses. [8,15] The common differential diagnoses in Indian population in adult to old age group are megaloblastic anemia, MDS and PNH. They have similar blood pictures and count except red blood cell morphology is severely macrocytic and hyperchromic (MCV>110fl and MCH> 35pg) in megaloblastic anaemia.[8] The megaloblastic anemia is nutritional anemia due to deficiency of Vitamin B12or Folic acid or combination of both. It is curable disease. Other common cause is drug induced marrow depression. Immunosuppressive therapy with cyclosporine and danogen, stanozolol, ATG and potential curative therapy with bone marrow transplantation are available in most parts of India. [5,9,10] Thus proper evaluation and diagnosis is imperative so that patients could be referred to PBSCT centres. Till such treatment is started, conservative treatment with random donor platelets (RDP), single donor platelets (SDP), packed red blood cells (PRBC) and antibiotics is done. However Cyclosporine and ATG are tried at referral hematology centres having support of blood bank, till financial and HLA–matched donor arrangement is made at BMT centres. [12,14,18]

## 5. Conclusion

Acquired Idiopathic aplastic anemia is major and serious health problem of India. The patients presenting with pancytopenia, must be evaluated by peripheral blood smear and bone marrow studies. Bone marrow trephine biopsy is diagnostic. The immunosuppressive to Peripheral blood stem cell transplantation is potentially curative modality of treatment. The data of our study is comparable with the international published data.

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