Epidemiology, Pathogenesis and Diagnosis of Aplastic Anaemia

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Epidemiology

The incidence of aplastic anaemia shows geographical variability. The incidence aplastic anemia varied from 10-52.7% among patients with pancytopenia. The incidence of severe and moderate AA was reported in 33.33% and 57.14% of cases respectively in from northern districts of West Bengal. One of the centres in India reported that aplastic anaemia accounted for 20-30% cases presenting with pancytopenia. The frequency of aplastic anaemia seen in hospitals of Asian countries is much higher than reported from the West, but the precise incidence of this disorder in India is not known. While incidence of aplastic anaemia (AA) in Europe and North America has been found to be low in prospective studies, reported as approximately 2 per million population per year in India and other Asian countries, it is about 2-3 times higher and could be as high as 6 to 8 per million population per year. The overall incidence of AA was 2.34 cases per million population per year in Barcelona and mortality at 2 years was nearly one death per million per year. Both incidence and mortality was shown to increase with age.

There was a biphasic age distribution with peaks between the ages of 15 and 25 years and a second smaller peak in incidence was noted after age 60 years with no significant difference in incidence between men and women. Aplastic anaemia affects people of all ages and all races. The variability in incidence rates in developing countries, is uncertain. However, exposure to environmental factors including viruses, drugs and chemicals, genetic background, diagnostic criteria, and study designs may be contributory. The median age of 8 years is consistently observed in various studies done from different regions. Recently, the frequency of Fanconi anaemia reported was 16.6% in Pakistan due to increased consanguinity, which is higher than reported in Western literature but similar to the studies from India.

Pathogenesis

A. Acquired Aplastic Anaemia

The pathogenesis of acquired AA is now believed to be immune mediated, with active destruction of haematopoietic stem cells (HSC) by lymphocytes, with activated type 1 cytotoxic T cells implicated. Recently, a causal relationship between haematopoietic stem cells (HSC) and microenvironment has been identified i.e. an abnormal expansion of suppressor T cells may cause depletion and possibly also clonal abnormalities of HSC. Clinically, it was found that a significant proportion of patients with acquired AA, ranging from 30% to 80%, given immnosuppressive therapy (IST) exhibit long-lasting recovery of peripheral blood counts supporting the hypothesis that responders would have immune-mediated suppression of haematopoiesis whereas non-responders could either have marrow failure caused by a primary HSC defect or immune-mediated aplasia with complete exhaustion of the stem cell pool. A further evidence in support of a primary immune-mediated pathogenesis of acquired AA comes from a recent well-designed study, signifying that CD4⁺CD25⁺FOXP3⁺ regulatory T cells are deficient in AA patients, similar to other autoimmune disorders. Thus, deficient regulation of T cells could then lead to an increase of T-bet protein levels in T cells, and increased interferon (IFN)-γ production. Polymorphism in cytokine genes may be associated with an increased immune response, including tumour necrosis factor...
The aberrant immune response and deficiencies
Severe radiation poisoning
rheumatoid arthritis
Autoimmune diseases like systemic lupus erythematosus and
Pregnancy
cytomegalovirus (CMV), parvovirus B19, and HIV
Other viral infections such as Epstein-Barr virus (EBV),
Hepatitis Infection
Benzene and other solvents
Anti-diabetics Chlorpropamide, Tolbutamide, and
Anti-depressants Dothiepin, Phenothiazines, Amphetamines
Anti-thyroids Carbimazole, Propylthiouracil
Anti-convulsants Phenytoin, Carbamazepine and valproic acid
Anti-malarials Chloroquine
Others Mebendazole, Thiázides, Allopurinol,
Mesalazine, Ticlopidine

Table 1 : Drugs which have been reported as a rare association with AA

<table>
<thead>
<tr>
<th>Category</th>
<th>Drugs</th>
</tr>
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<tbody>
<tr>
<td>Anti-inflammatory</td>
<td>Nonsteroidal anti-inflammatory drugs (NSAIDS) - Indomethacin, Diclofenac, Naproxen, Piroxicam, Phenylbutazone</td>
</tr>
<tr>
<td></td>
<td>Disease Modifying Anti-Rheumatic Drugs (DMARD) - Gold, Penicillamine, Sulphasalazine</td>
</tr>
<tr>
<td>Antibiotics</td>
<td>Chloramphenicol, Sulphonamides, Cotrimoxazole, Linezolid</td>
</tr>
<tr>
<td>Diuretics</td>
<td>Furosemide, Thiázides</td>
</tr>
<tr>
<td>Anti-convulsants</td>
<td>Phenytoin, Carbamazepine and valproic acid</td>
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Table 2 : Potential aetiological agents in AA (occupational and environmental exposures)

<table>
<thead>
<tr>
<th>Category</th>
<th>Agents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzene and other solvents</td>
<td></td>
</tr>
<tr>
<td>Hepatitis Infection</td>
<td></td>
</tr>
</tbody>
</table>
| Other viral infections    | as enlisted in Table 1 or viral infection and perhaps endogenous antigens generated by genetically altered bone marrow cells. Environmental triggers are linked to exposure to drugs, viruses and toxins (benzene, pesticides and other chemicals) but most cases (70–80%) are idiopathic, which leads to marrow failure is a severe idiosyncratic complication. Certain histocompatibility locus specificities, especially HLA DR2, are associated with an underlying predisposition to acquired aplastic anaemia. The incidence of aplastic anaemia is subjected to wide variation throughout the world, the reason apparently lying in the environmental factors as mentioned in Table 2 rather than genetic factors. A striking example was the large aetiologic fraction in a rural region accounted for by animal fertiliser.

Table 3 : Other risk factors

<table>
<thead>
<tr>
<th>Category</th>
<th>Agents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agricultural pesticides</td>
<td>Organochlorines e.g. Lindane, Organophosphates, Pentachlorophenol</td>
</tr>
<tr>
<td>Cutting oils and lubricating agents</td>
<td></td>
</tr>
<tr>
<td>Non-bottled water, non-medical needle injury, farmers exposed to ducks and geese, animal fertiliser</td>
<td></td>
</tr>
</tbody>
</table>

Many drugs and chemicals have been implicated in the aetiology of aplastic anaemia, but for only very few is there reasonable evidence for an association from case control studies, and even then it is usually impossible to prove causality. A careful drug history should be obtained, detailing all drug exposures for a period beginning 6 months and ending 1 month prior to presentation.

AA can follow specific viral infections, as in post-seronegative hepatitis. Post-hepatitis AA syndrome accounts for about 10% of marrow failure in Western case series. Hepatitis associated AA was seen in 21% of cases. AA is also a rare complication of pregnancy. Other risk factors for AA, apart from these have been listed in Table 3.

Inherited aplastic anaemia

a. Fanconi anaemia : Congenital aplastic anaemia is rare, the commonest type being Fanconi anaemia, that leads to bone marrow failure. It is primarily an autosomal recessive disorder. Till date 16 FA or FA-like genes have been discovered. These genes account for over 95% of all known FA patients. Some patients do not appear to have mutations in these 15 genes, so we anticipate that additional FA genes will be discovered in the future. FA occurs equally in males and females. It is found in all ethnic groups. The current median lifespan for a patient with FA is 33 years, although there are now patients living into their 30s, 40s and 50s. Though considered primarily a blood disease, it can affect all systems of the body. Many patients eventually develop acute myeloid leukaemia (AML) at a very early age. FA patients are extremely likely to develop a variety of cancers and at a much earlier age than patients in the general population.

b. Dyskeratosis congenita : Dyskeratosis congenita (DC), an X-linked inherited disorder arising as a consequence of short telomeres and mutations in telomere biology is characterised by a classic triad of dysplastic nails, lacy reticular pigmentation of the upper chest and/or neck, and oral leukoplakia. Production of the altered protein dyskerin, leads to vulnerable skin, nails, and teeth which lead to higher permeability for noxious agents which can induce carcinogenesis. The incidence of dyskeratosis congenita is again rare, with a prevalence of 1 in 40,000 newborns. A recent study has shown that the incidence may be even lower, with only 1 in 100,000 newborns. Dyskeratosis congenita is a rare disorder that affects the skin, nails, and oral cavity. The characteristic triad of skin pigmentation, nail dystrophy, and oral leukoplakia is seen in patients with dyskeratosis congenita. The condition is caused by mutations in the telomerase reverse transcriptase (TERT) gene, which leads to short telomeres and increased chromosomal instability. Dyskeratosis congenita is associated with an increased risk of skin and oral cancers, as well as other malignancies. It is a life-long condition, and patients require regular monitoring and intervention to prevent complications. The condition is often diagnosed in childhood or adolescence, and many patients develop cancer in adulthood. The diagnosis of dyskeratosis congenita is usually made based on clinical features and genetic testing. Treatment is primarily supportive, with treatments focused on managing symptoms and preventing complications. In some cases, bone marrow transplantation may be considered as a curative treatment option.
c. Other causes of inherited aplastic anaemia: Shwachman-Diamond syndrome

This is also a rare congenital disease caused by abnormal copies of a gene called SDS. Here, the major problem is poor production of white blood cells, although the other cell lines can also be abnormal. In both of these, patients will often have other problems such as short stature and other bone abnormalities.

### Diagnosis of Aplastic Anaemia

Despite the precision of its diagnostic criteria, aplastic anaemia has always been a diagnosis of exclusion. No single test allows us to reliably diagnose idiopathic aplastic anaemia. Consequently, the diagnostic evaluation has become increasingly detail driven in its attempt to exclude a list of potential alternative aetiologies of BM failure. Figure 2 enlists the various diagnostic criteria of AA in correlation with the etiology.

It remains essential to obtain a thorough history and perform a detailed examination. One goal of history taking is to elicit evidence of any drug or toxin exposures that have been associated with BM aplasia. Physical examination includes looking for morphologic abnormalities that are characteristic of

### Table 4: Definition of severity of AA based on CBC and bone marrow

<table>
<thead>
<tr>
<th>Classification</th>
<th>Criteria</th>
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<tbody>
<tr>
<td>Severe</td>
<td>BM Cellularity &lt; 25% (or &lt; 50% if &lt; 30% of BM is haematopoietic cells) and &gt; 2 of the following:</td>
</tr>
<tr>
<td></td>
<td>• Peripheral blood neutrophil count &lt; 0.5 x 10^9/L</td>
</tr>
<tr>
<td></td>
<td>• Peripheral blood platelet count &lt; 20 x 10^9/L</td>
</tr>
<tr>
<td></td>
<td>• Peripheral blood reticulocyte count &lt; 0.5 x 10^9/L</td>
</tr>
<tr>
<td>Very Severe</td>
<td>As above, but peripheral blood neutrophil count must be &lt; 20 x 10^9/L</td>
</tr>
<tr>
<td>Non-severe</td>
<td>Hypocellular BM with peripheral blood values not meeting criteria for severe aplastic anaemia</td>
</tr>
</tbody>
</table>

### Fig. 1: Etiology and Diagnosis of Aplastic Anaemia

- **Aetiology**
  - I. Idiopathic
  - II. Exposure to Chemicals
  - III. Licensed Drugs
  - IV. Infections, Pregnancy, Auto-immune disorders, Radiation Poisoning

- **Bone marrow failure with reduced production of**
  - Red blood cells
  - White blood cells
  - Platelets

- **Symptoms due to**
  - Anaemia
  - Thrombocytopenia leading to bleeding
  - Infection

- **History:** Exposure to-
  - Toxic drugs
  - Environmental chemicals
  - Infections
  - Radiation

- **Physical Examination**
  - Anaemia
  - Bruising
  - Bleeding
  - Fever
  - Enlarged lymph gland
  - Splenomegaly

- **Laboratory Investigations**
  - Full blood count and film may show:
    - Anaemia
    - Reduced or abnormal white cells
    - Reduced platelets

- **Bone marrow examination may show features of**
  - Leukaemia
  - Aplasia or hypo aplasia
  - Lymphoma
  - Malignant Infiltrations
  - Infective Infiltrations

- **Further investigations**
  - Requires specialist advice and facilities
The pathophysiology of AA includes a number of cellular and molecular pathways involving both effector (T cells) and target (at haematopoietic stem and progenitor) cells. Antigens are presented to T cells by antigen-presenting cells (APCs), which trigger T cells to activate and proliferate. Increased production of interleukin-2 leads to the polyclonal expansion of T cells. An immunological cascade results in the production of a number of mediators and toxic effects, leading to reduced cell cycling and cell death by apoptosis, and ultimately resulting in bone marrow failure.

Fanconi anaemia and Dyskeratosis congenita. Acquired AA is generally not associated with lymphadenopathy and organomegaly.

Investigations required for the Diagnosis of AA

1. Complete Blood Count, reticulocyte count, peripheral smear: The complete blood count (CBC) typically shows pancytopenia although usually the lymphocyte count is preserved. In most cases the haemoglobin level, neutrophil and platelet counts are all uniformly depressed, but in the early stages isolated cytopenia, particularly thrombocytopenia, may occur. For a diagnosis of AA, there must be at least two of the following:
   - Haemoglobin level < 100 g/L
   - Platelet count < 50 x 10^9/L
   - Neutrophil count < 1.5 x 10^9/L

Anaemia is accompanied by reticulocytopenia, and macrocytosis is commonly noted. In aplastic anaemia, neutrophils may show toxic granulation and anisopoikilocytosis is common. Blood smear examination is important to exclude the presence of dysplastic neutrophils and abnormal platelets, blasts and other abnormal cells, such as hairy cells (as seen in hairy cell leukaemia). Platelets are reduced in number and mostly of small size.

2. Bone marrow aspirate and trephine biopsy examination: Both a bone marrow aspirate and trephine biopsy are required. Bone marrow aspiration and biopsy may be performed in patients with severe thrombocytopenia without platelet support, providing that adequate surface pressure is applied. Fragments are usually readily obtained from the aspirate. Difficulty obtaining fragments should raise the suspicion of a diagnosis other than aplastic anaemia. The fragments and trails are hypocellular with prominent fat spaces and variable amounts of residual haematopoietic cells. Megakaryocytes and granulocytic cells are reduced or absent. Lymphocytes, macrophages, plasma cells and mast cells appear prominent. A trephine is crucial to assess overall cellularity, to assess the morphology of residual haemopoietic cells and to exclude an abnormal infiltrate. In most cases the trephine is hypocellular throughout but sometimes it is patchy, with hypocellular and cellular areas. Thus, a good quality trephine of at least 2 cm is essential. A ‘hot spot’ in a patchy area may explain why sometimes the aspirate is normocellular. Care should be taken to avoid tangential biopsies as subcortical marrow is normally ‘hypocellular’.
Focal hyperplasia of erythroid or granulocytic cells at a similar stage of maturation may be observed. Sometimes lymphoid aggregates occur, particularly in the acute phase of the disease or when the aplastic anaemia is associated with systemic autoimmune disease, such as rheumatoid arthritis or systemic lupus erythematosus. The reticulin is not increased and no abnormal cells are seen. Increased blasts are not seen in aplastic anaemia, and their presence either indicates a hypocellular MDS or evolution to leukaemia. Severity of AA based on CBC and bone marrow has been defined in Table 4.

3. Liver function tests and viral studies: Liver function tests should be performed to detect antecedent hepatitis. Blood should be tested for hepatitis A antibody, hepatitis B surface antigen, hepatitis C antibody, Epstein–Barr virus (EBV) and Cytomegalovirus (CMV). Parvovirus causes red cell aplasia but not aplastic anaemia. Human immunodeficiency virus (HIV) is not a recognised cause of aplastic anaemia, but it can cause isolated cytopenias.

4. Tests to detect a PNH clone: Paroxysmal nocturnal haemoglobinuria should be excluded by performing flow cytometry. Evidence of haemolysis associated with PNH should be quantified with the reticulocyte count, serum bilirubin, serum transaminases and lactate dehydrogenase (LDH).

5. Screen for inherited disorders: Peripheral blood lymphocytes should be tested for spontaneous and diepoxybutane (DEB) or mitomycin C (MMC)-induced chromosomal breakage (stress cytogenetics) to identify or exclude Fanconi anaemia. Dyskeratosis congenita may be excluded by identifying a known mutation but there are probably many mutations yet to be identified. Along with measuring telomere lengths, this is not currently available as a routine clinical service.

6. Radiological investigations: A chest X-ray is useful at presentation to exclude infection and for comparison with subsequent films. Abdominal ultrasound: the findings of an enlarged spleen and/or enlarged lymph nodes raise the possibility of a malignant haematological disorder as the cause of the pancytopenia. In younger patients, abnormal or anatomically displaced kidneys are features of Fanconi anaemia.

References


