NEXT BIOBANK

Netcells Stem Cells
Use of cord blood stem cells for treatment of Aplastic Anaemia.

What is Aplastic Anaemia

Aplastic anemia (AA) is a clinical and pathological entity of bone marrow failure that causes progressive loss of haematopoietic stem cells (HSC), resulting in pancytopenia. AA can be inherited or acquired and patients may present along a spectrum of symptoms, ranging from incidental findings on peripheral blood tests to life-threatening neutropenic infections or bleeding. The pathophysiology and treatment approach vary significantly depending on the cause. Therefore, recognition of the type of marrow failure disease is critical in establishing the management plan.

Aetiology of Aplastic Anaemia

AA is a rare disorder, with an incidence of between 1.5 (in European populations) and 7 cases (in Asian populations) per million individuals per year. 80% of all cases of AA are acquired. The initial incidental peak is at 15-25 years of age, while the second generally occurs beyond 60 years of age. The leading hypothesis as to the cause of most cases of acquired AA is that a dysregulated immune system destroys HSCs. The expected causes are drugs (such as cytotoxic, anti-inflammatory and anti-thyroid drugs and antibiotics); chemicals such as benzene; viruses (including Epstein-Barr virus (EBV), Hepatitis variants – non-A,B,C,D,E and G, HIV and Parvo Virus); autoimmune disorders and radiation. Remaining cases are inherited and linked to a diagnosis of Fanconi Anaemia, Paroxysmal Nocturnal Haemoglobinuria (PNH) and Myelodysplastic Syndromes. In these instances, AA develops within the first decade of life.

Pathophysiology of Aplastic Anaemia

There are 3 categories that cause marrow failure in patients with AA:

1. Immune-mediated destruction of the bone marrow

It is believed that the majority of acquired causes of AA result from immune-mediated responses. There are two different immune-mediate responses that cause a pancytopenia:

1. Historically, the understanding of acquired AA implicates cytotoxic T-lymphocyte–mediated destruction of CD34+ haematopoietic stem cells (HSCs) with the down-regulation of T regulator cells and aberrant cytokine profiles. This hypothesis served as the basis for treatment of acquired AA with immunosuppressive therapy, predominantly anti-thymocyte globulin (ATG) combined with cyclosporine A.

2. The second response is by activated complement. Patients with AA often harbour progenitor cell clones associated with PNH. PNH clones have been identified in more than 50% of patients with AA. PNH represents a clonal disorder of haematopoiesis in which cells harbour X-linked somatic mutations in the PIGA gene; this gene encodes a protein responsible for the synthesis of glycosylphosphatidylinositol anchors on the cell surface. The lack of these cell surface proteins, specifically CD55 (also known as decay accelerating factor) and CD59 (also known as membrane inhibitor of reactive lysis), predisposes red cells to increased complement-mediated lysis. The exact mechanism for the development of these clones in patients with AA is not fully understood and patients with AA may develop PNH clones, while conversely patients with PNH may develop AA.

2. Constitutional / inherited syndromes

The inherited marrow failure syndromes (IMFSs) are a group of disorders characterized by cellular maintenance and repair defects, leading to cytopenia's, increased cancer risk, structural defects, and risk of end organ damage, such as liver cirrhosis and pulmonary fibrosis. The most common diseases include Fanconi Anaemia, dyskeratosis congenita/telomere biology disorders, Diamond-Blackfan Anaemia, and Shwachman-Diamond Syndrome, but with the advent of whole exome sequencing, new syndromes continue to be discovered. All the above syndromes result from germline mutations. While classically these disorders present in children, adult presentations are now commonplace.

Broadly, the pathophysiology of inherited AA relates to the defective HPSCs and an accelerated decline of the HSC compartment. The most common IMFSs, Fanconi Anaemia and telomere biology disorders, are associated with numerous mutations in DNA damage repair pathways and telomere maintenance pathways. The implications of early diagnosis of an IMFS lie in the approach to treatment and prognosis.

3. Direct Marrow Damage

Marrow damage occurs most often from chemotherapy and radiation. Marrow effects are dose-dependent and, at conventional doses, are transient. Benzene, an inexpensive solvent, also damages hematopoiesis, and industrially-exposed workers figured prominently in the early literature of AA. Benzene now is a negligible risk factor in most countries, however, in China, which is rapidly industrializing and less regulated, benzene remains a more common workplace toxin.

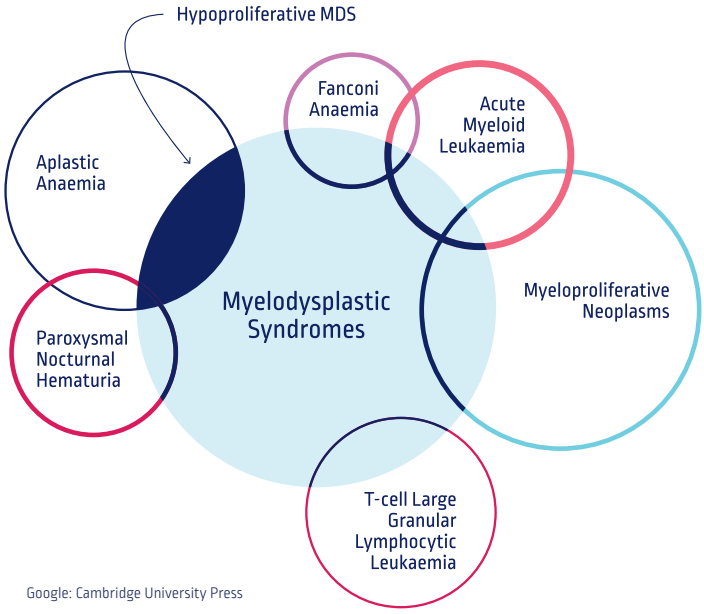
Differential Diagnosis

Because of the over-lapping disease pathology, differential diagnosis is important. Myelodysplastic Syndrome (MDS) and secondary Acute Myeloid Leukemia (AML) are two clonal disorders that may arise from a background of AA. Hypoplastic MDS can be difficult to differentiate from AA at diagnosis based on morphology alone, although recent work has demonstrated that molecular testing for somatic mutations can aid in differentiating a subset of AA patients who are more likely to progress to MDS.

Differential diagnosis can be done based on:

- Clinical presentation: Fanconi Anaemia presents with stigmata and stunted growth and PNH presents with dark morning urine due to complement mediated haemolysis and thrombosis.
- Laboratory tests (cytogenetics and immunophenotyping) which include screening of 50 genes that cause constitutional marrow failure including PIG-A (PNH), Karyotyping – DNA breakages (Fanconi Anaemia) and flow cytometry: CD55/CD59.
- Lifestyle history to determine exposure to benzene, chemo and radiation to cause pancytopenia.

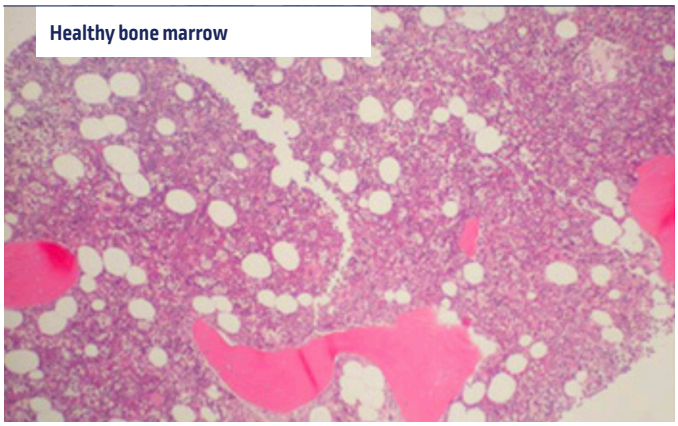
Differential Diagnosis

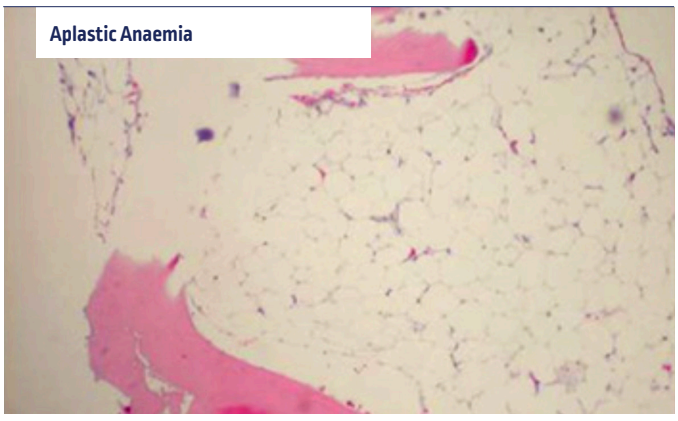


Google: Cambridge University Press

Diagnosing AA

With a full blood count, patients with AA will present with haemoglobin below 10g/dl; low total leukocyte count, low platelet count, a low whole blood cell count and a low reticulocyte index. A bone marrow aspirate will be aplastic with fat spaces, flow cytometry will show a low CD55/CD59 ratio and a peripheral blood smear will show no abnormal cells/blasts and NNA.





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Treatment of AA

Severe AA can be a particularly aggressive with a poor prognosis. If an HLA-matched donor can be found, then generally patients can be treated with an HSC transplant to replace their defective bone marrow. Given the challenges associated with finding a donor, immunosuppressive therapy (IST) is an option that has been shown in previous studies to have good efficacy. This involves a combination of rATG which is an immune globulin that depletes T cells (reduces abnormal T cell activity) and CsA which is a calcineurin inhibitor that reduces T cell activation and proliferation; with no inhibiting effect on T regulation. This combination has been shown to improve survival, however is still plagued by patient relapse in 20-40% of patients.

The use of umbilical cord blood (UCB) is an excellent alternative to bone marrow as CB contains a significant number of HSCs as well as progenitor cells and regulator T cells. These all have crucial roles to play in immune reconstitution in a patient with AA. In addition, cord blood is known to have reduced immunogenicity (compared to bone marrow) which contributes to better engraftment, when compared to BM. Cases of graft versus host disease are also significantly less.

Case Studies

The first study, *efficacy and safety of combined immunosuppressive therapy plus umbilical cord blood infusion in severe aplastic anemia patients· a cohort study*,[1] investigates the efficacy and safety of combining immunosuppressive therapy (IST) and UCB infusion to treat patients with severe AA. 68 patients, older than 2 years of age and with a weight >12 kg, diagnosed with severe AA were included in the study. According to the Camitta criteria, severe AA is defined as having a bone marrow cellularity of less than 30% and at least 2 of the following:

+ Absolute neutorphil count of less than 0,5 billion cells/ litre
+ Platelet count less than 20 billion cells/ litre
+ Reticulocyte count less than 20 billion cells/ litre

There were several common adverse events, including fever, rash, infection, haemorrhage and ATG-associated serum disease, however UCB did not significantly increase the number of these adverse events.

Results showed that patients treated with a combination of both IST and UCB infusion observed favourable treatment responses. They had a better and quicker recovery of ANC and platelets compared to the IST group as well as a better survival rate. The results also suggested that a combination of both IST and UCB can be used effectively and safely. However, this study was done on a relatively small sample and further studies should be carried out with larger sample sizes.

In the second study, *Umbilical cord blood transplantation supplemented with the infusion of mesenchymal stem cell for an adolescent patient with severe aplastic anemia· a case report and review of literature*,[2] mesenchymal stem cells (MSCs) have been found to support haematopoiesis and facilitate immunomodulation and secrete pro-regenerative factors. As such they are regarded as potential therapeutic agents in the treatment of haematological and immunological disorders and are used in the treatment of auto-immune diseases.

UCB transplantations are hampered by the delayed HSC recovery, so in this case study a 13 year old girl with classic aplastic anaemia symptoms received 33 ml of UCB from her sibling sister in 2013. However, there was no engraftment and her condition was not seen to improve. The MSCs from cord tissue were then obtained and the patient received 20 million MSC cells.

The results showed that better engraftment with better tolerance to host and donor antigens were achieved by addition of the MSCs. The MSCs helped by exhibiting immune suppressive activity on T-cells, B cells and natural killer cells. This study indicated that the UCB treatment alone was not sufficient and a combination of UCB and MSC improved the condition. This is a single case, and further controlled trials are required.

References

1. Luo X, et al. Efficacy and safety of combined immunosuppressive therapy plus umbilical cord blood infusion in severe aplastic anemia patients: a cohort study. Exp Ther Med. 2018; 15:1966-1974.
2. Luan C, et al. Umbilical cord blood transplantation supplemented with the infusion of mesenchymal stem cell for an adolescent patient with severe aplastic anemia: a case report and review of literature. Patient Prefer Adherence. 2015; 9: 759–765.