Review Article

The Scope of Aplastic anaemia: etiology, pathophysiology, pharmacotherapy and pharmacoeconomic impact in clinical patient management.

ABSTRACT

The blood disease called aplastic anaemia (AA) isconsidered a rare blood condition known to be in some occasion to be a public health concern, as a life-threatening health accounting for the major causes of bone marrow failure. AA may provoke bone marrow hypoplasia or aplasia in patients and could also lead to anaemia and can cause pancytopenia. The most likely immediate cause of AA is an autoimmune response of T lymphocytes against hematopoietic stem cells or in some cases cause a congenital defect or acquired damage to blood cells leading to inhibition of their cell division and eventual differentiation. AA can easily develop within a few days then slowly evolves for several weeks or months. The signs and symptoms of AA includes anaemia, neutropenia, and thrombocytopenia. The continuous improvement in AA treatment strategies of patients, there is a progressive better outcomes of both family and unrelated donor haematopoietic stem cell transplantations (HSCTs), coupled with a better revised protocol of immunosuppressive therapy (IST). The treatment protocol approaches for AA depend mainly on three main factors such as the age of the patient and matched siblings' donor, the severity of the disease. All patients diagnosed with AA are in need of an appropriate supportive treatment care and monitoring platform that is adapted to the current clinical settings. Supportive treatment is recommended both before, during and after invasive causal treatment that mainly concern the transfusion of leukocyte-depleted blood components, the use of anti-infectious prophylaxis or treatment of infections of the patients. In most circumstances, supportive therapy is the sole therapeutic regimen option, mostly in elderly patients presented with comorbidities. The objective of this work is to present a comprehensive review of the scope and concepts of AA within the framework of the etiology, pathophysiology, therapeutic options, treatment complications and the pharmaco-economics implication of patients' treatment and management.

Keywords: Aplastic anaemia; etiology, pathophysiology, pharmacotherapy, clinical presentation; pharmacoeconomics

1. INTRODUCTION

The blood disease called aplastic anaemia (AA) is a report rare bone marrow disorder characterized by pancytopenia [1]. It may be considered to be congenital, idiopathic, but in some seldom cases are known to be caused by some drugs, xenobiotics and infectious pathogens [2]. Patients can be diagnosed with hypocellular bone marrow which can most likely be treated by an allogenic haematopoetic stem cell transplant. Recommendation of supportive care of patients with effective transfusion and immunosuppressive therapy may improve symptomatic relief and better quality of life for the patients [1-3]. AA is known to occur when there is acquired or

congenital bone marrow failure in the production of all cell lines, without the presence of cancerous invasions and fibrosis, that can result to pancytopenia. This disease has rare occurrence with respect to the multiple challenges faced with diagnosis process, coupled with the lack of limited access to treatment in low income countries. [4]. The progress in the discovery and development of better therapeutic options has been reported, most especially in cases of children with severe pancytopenia. However, results of specialist therapy are geared towards the targeted supportive care. The incidence of AA developed countries are estimated at about two to three cases/million/year and in and in Asia about five to six cases/million/year [4, 5]. AA may occur at any age, but in most cases can range from 15 to 25 years and over 60 years, with no consideration for gender or race diversity, since its etiology has been documented [5, 6].

Pancytopenia can occur when there is a problem with the blood-forming stem cells in the bone marrow, rather than anaemia. The disease is estimated to occur in two to four individuals per million populations every year [2, 8]. Paul Ehrlich elucidated the concept of aplastic anemia in 1888, when he studied a case of a pregnant woman who died of bone marrow failure [9]. It was only until 1904 that Anatole Chauffard named this disorder aplastic anemia [7, 10].

1.1. Etiology of AA.

AA could result in the reduction in the pluripotent stem cell number below a critical threshold mass due to a conflict between self-renewal versus differentiation leading to an ultimate stem cell or bone marrow failure as illustrated in figure 1. There have been reports in a complex balance between stem cell differentiation and regeneration responsible for the etiology of aplastic anemia [2, 8]. Several cases reported indicates that AA is considered idiopathic in nature, even though it can be linked to cytotoxic drugs (chloramphenicol, gold) [9], radiation, toxic compounds such as benzene, solvents and glue vapours, and viruses like the viral infections Epstein-Barr Virus Infection, and Seronegative Non A-G hepatitis [11]. For immune-related disorders we have the Eosinophilic fasciitis, SLE, Graft versus the host disease [10]. Other conditions reported include thymoma, anorexia nervosa and paroxysmal nocturnal haemoglobinuria (PNH) [11, 12]. The complex balance between stem cell differentiation and regeneration in the etiology of aplastic anemia has been illustrated in figure 1.

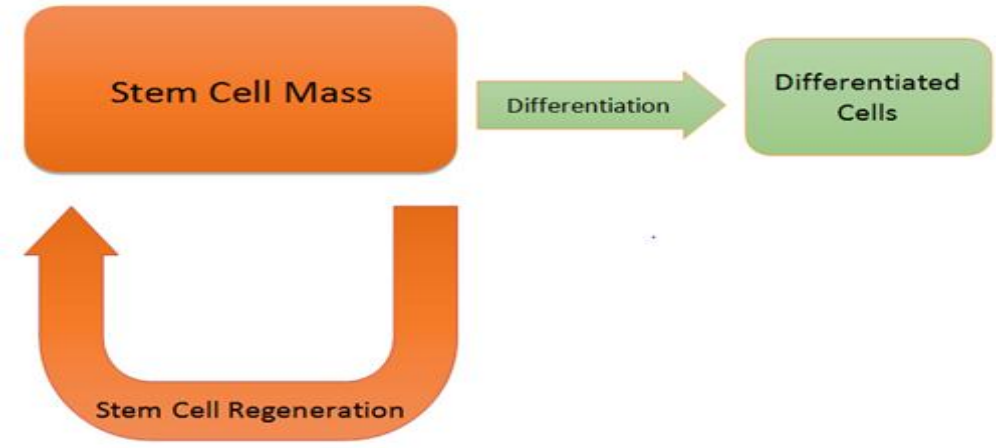

Figure 1. Illustration of the complex balance between stem cell differentiation and regeneration in the etiology of aplastic anemia [2]].

1.2. Drug induced Aplastic anaemia

The reported cases of patients exposed to cytotoxic drugs do not develop AA, and the specific mechanism action for the idiosyncratic reaction is not well documented. The P-glycoprotein, the Multi-Drug Resistance Gene, (MDR-1) gene product, and the multidrug resistance-associated protein (MDR-AP), are considered as energy-dependent transmembrane efflux pumps for a wide range of lipophilic drugs [2, 11]. They play a major role in keeping the drugs out of the cells as illustrated in figure 2, which explains the mechanism of drug efflux from the cell [12]. An upregulation of the P-glycoprotein predisposes the cancer cells a multidrug resistance phenotype, whereas downregulation in normal cells renders the cytoplasmic accumulation of drugs and increases their toxic effects [13-14].

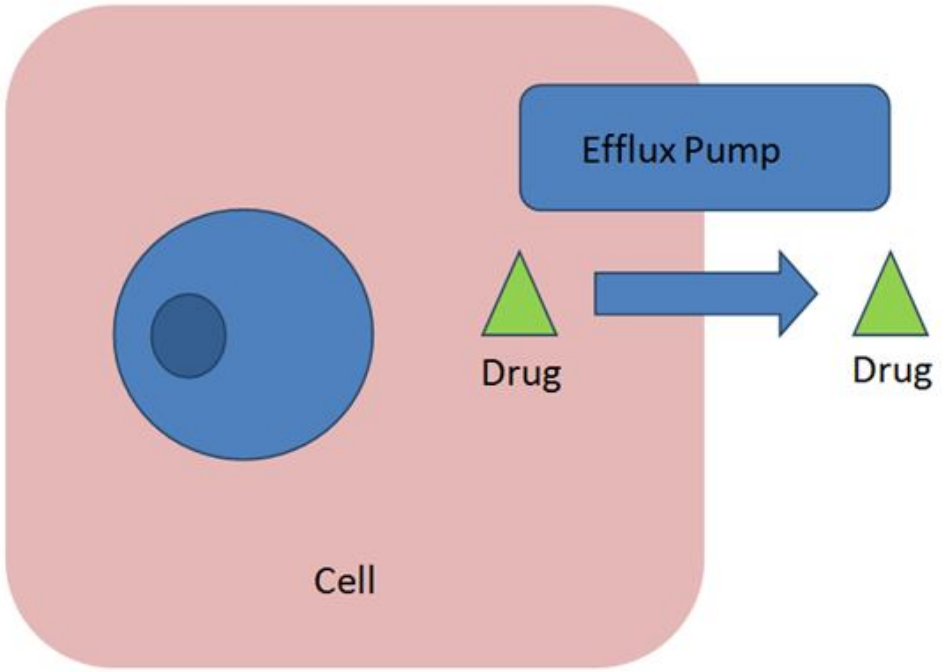

Figure 2: Illustration of the mechanism of drug efflux from the cell [2].

1.3. Aplastic Anaemia caused by Viral infections

Certain viruses are known to cause AA as indicated.

1.3.1. Parvovirus B19

This virus generally attacks the pro-erythroblasts and provoke transient red cell aplasia, that has been shown in patients with chronic haemolytic anaemia [12]. On the other hand, pancytopenia is mostly seen in patients with immune-compromised conditions [3, 14]. Human parvovirus B19 has been linked to a wide spectrum of diseases such as the *Erythema infectiosum* (EI) in pediatric cases. One of the most recorded complications of parvovirus B19 infection is the transient aplastic crises in patients with chronic haemolytic anaemia, such as the sickle cell disease and hereditary spherocytosis [15]. Pure red cell aplasia may in some cases develop with a chronic infection of parvovirus B19 in immunocompromised patients [15]. On the other hand, parvovirus B19 infection has been reported to be linked to idiopathic *Thrombocytopenic purpura* and neutropenia [16, 17]. Of recent, a case of severe aplastic anaemia (SAA) has been reported in a previously healthy boy without any underlying diseases, following asymptomatic infection with parvovirus B19 [16]. Studies on the frequency of parvovirus B19 infection in paediatric cases with SAA has been reported in order to explore the association between parvovirus B19 infection and aplastic anaemia [17, 18].

1.3.2. Hepatitis virus and human immunodeficiency virus (HIV)

These viruses for their action implicate the T cell activation with the release of cytokines or the activation of cytotoxic T cell clones which can recognize similar target antigens on the liver and the bone marrow cells [19, 20]. Hepatitis-associated aplastic anaemia (HAAA) is a well-known variant of the clinical syndrome, acquired aplastic anaemia, in which an acute attack of hepatitis can lead to bone marrow failure and pancytopenia [20-21]. HAAA was first reported in two patients' cases by Lorenz and Quaiser in 1955 [21] and since then the number of cases increased to 200 in 1975 and now many cases are reported [22]. However, this HAAA syndrome has only been reported in 2-5% cases in developed countries and 4-10% for low income countries, like in areas with increased prevalence of hepatitis and Human Immunodeficiency Viruses (HIV), in the Far East Asia [22], with low socioeconomic status [19]. HAAA occurrence is not linked to relative age, sex and severity of hepatitis. Currently more report has been found in children, adolescent boys and in adolescent men [23].

1.3.3. Aplastic Anaemia linked to viral hepatitis

The pathophysiology of AA is immune-mediated in most reported cases, and are activated by T1 lymphocytes identified as effector cells. The disease has been successfully treated with haematopoietic stem cell transplantation [23, 24]. Syndrome of bone marrow failure can occur following the development of acute seronegative hepatitis, and this syndrome in most cases affects young males who present severe pancytopenia two to three months after an episode of acute hepatitis [24]. The clinical course of hepatitis is commonly benign in nature, and the bone marrow failure could severe and usually fatal if treated is not done early enough. There have been no correlations reported to show association between the severity of hepatitis and AA [25]. The characteristics and response of AA to immunotherapy indicate a significant role of immune mechanism in the pathogenesis of HAA.

The main target organ of the immune response is the liver as shown by the result of the time interval between hepatitis and failure [26]. Liver histology is characterized by T cells invasion of the parenchyma as reported in acute hepatitis. Recently in HAA, it has been shown that intra-hepatic and blood lymphocytes with T cell repertoire similar to that of expanded T cell clones return to a normal distribution after response to immunosuppressive treatment, suggesting the antigen or T cell clearance. Therapeutic options are the same as in acquired aplastic anaemia [27, 28].

1.4. Auto-immune response to aplastic anaemia

Acquired idiopathic aplastic anemia (IAA) is a rare haematologic disorder that is characterized by the failure of haematopoiesis secondary to an immune-mediated damage of the bone marrow. IAA is generally considered as an auto-immune disease with a T-cell-mediated pathophysiology [29]. The oligoclonal pattern of effector memory $CD8^{+}CD57^{+}$T cells in IAA patients has been described using a combined deep sequencing and flow cytometry option [30]. It is reported that clonally expanded T-cell populations are easily detectable within the effector memory compartment, and that they tend to correlate with disease activity. Therefore, the characterization of the T-cell receptor (TCR) signals by a high-resolution technique could play a significant part to confirm the diagnosis of immune-mediated IAA and the monitoring of affected patients during their disease development [31].

Clinical and experimental evidence are widely availabe to support the autoimmune pathophysiology of IAA [31]. The most evident evidence is that patients with IAA may respond to T- cell-targeted immunosuppressive therapies (IST), with rates of haematologic responses ranging between 50% and 70% [24, 32]. Investigations in the past two decades have demonstrated a lot of experimental data that support the hypothesis of an immune-mediated pathophysiology. Increased in circulating activated T cells has been described in IAA patients in the ‘80s [26, 33]., that are known to suppress haematopoiesis by secreting different inflammatory cytokines [34], or through the cell-mediated direct killing. Within the different inhibitory cytokines, the interferon-γ (IFN-γ) plays a significant role in the suppression of human hematopoietic stem cells (HSC) *in vivo*, and also as suggested by *in vitro* inhibition of cell cycle progression and induction of apoptosis of haematopoietic signals [26, 35]. It has been shown that IFN-γ may exert its inhibitory response on HSC, preventing the homeostatic survival signal delivered by thrombopoietin through its cognate receptor c-MPL [27, 36]. This inhibitory milieu is generated by immune cells, of mostly by T cells that can be activated and proliferate in response to an antigen-driven stimulation. As the search for these putative antigens remains unsuccessful, the demonstration of clonal expansion of T-cell populations identified by their TCR has been considered a strong evidence of a T-cell-mediated pathophysiology in IAA [28].

Autoimmune bone marrow inhibition may be mediated by the release of interferon gamma (IFN-gamma) due to its marrow suppressing effect, this is influenced by the transcription factor T-bet or cytokines such as TNF-alpha and other interleukins [27, 28]. Nonregulated lymphocyte activation, like mutations of perforin in haemophagocytic lympho-histiocytosis, or an autoimmune state due to impaired number or functions of the cluster of differentiation (CD4+/CD25+)/ transcription factor fork-head box P3 positive (FOXP3+) T regulatory cells, secondary to the actions of T helper 17 (Th17) cells could lead to a haematopoietic inhibitory

response. This inhibition may be mediated by IFN-gamma or the cytokine cascade that is released by IFN-gamma and can result in apoptotis of haematopoietic stem cells in the bone marrow [28-30].

2.0. CLINICAL MANIFESTATIONS

Patients with AA appears well, before any diagnosis. However, patients may seek medical attention due to fatigue and other symptoms associated with progressive anaemia. More common manifestations include recurrent infections due to profound neutropenia or mucosal haemorrhage caused by thrombocytopenia [31-35]. Infections are frequently are of bacterial nature although invasive fungal infection are recorded as a common cause of death; particularly in patients with chronic and severe neutropenia [37, 38]. Increased and constant menstrual flow is one of the common complaints in premenopausal women. AA patients can express no symptoms but when present, the signs and symptoms can include: Shortness of breath, Rapid or irregular heart rate Fatigue, Pale skin, Frequent and prolonged infections, Other unexplained effects are known such as easy bruising, nosebleeds and bleeding gums, prolonged bleeding from cuts, skin rash, dizziness, headache, and fever [36-40]. Aplastic anemia can be short-lived, or can become chronic [39, 40], severe and in some cases even fatal [41].

2.1. Diagnosis

Patients with AA, have clinical findings correlated with pancytopenia, especially pallor and petechiae. The liver, spleen or lymph nodes are generally not enlarged [42]. A complete blood count with differential, bone marrow aspiration and biopsy with the measurement of red cell membrane or neutrophil CD59 by flow cytometry, and cytogenetics are indicated [34, 43]. Diagnosis of AA is established by showing evidence of pancytopenia and hypocellular bone marrow. An important differential diagnosis is hypoplastic myelodysplastic syndromes (MDS), which should be confirmed due to significant differences in management and prognosis [44]. The etiologies of the aplastic anaemia and the main diagnostic characteristics of aplastic anemia and hypoplastic myelodysplastic syndromes are illustrated in figure 3 and table 2 respectively

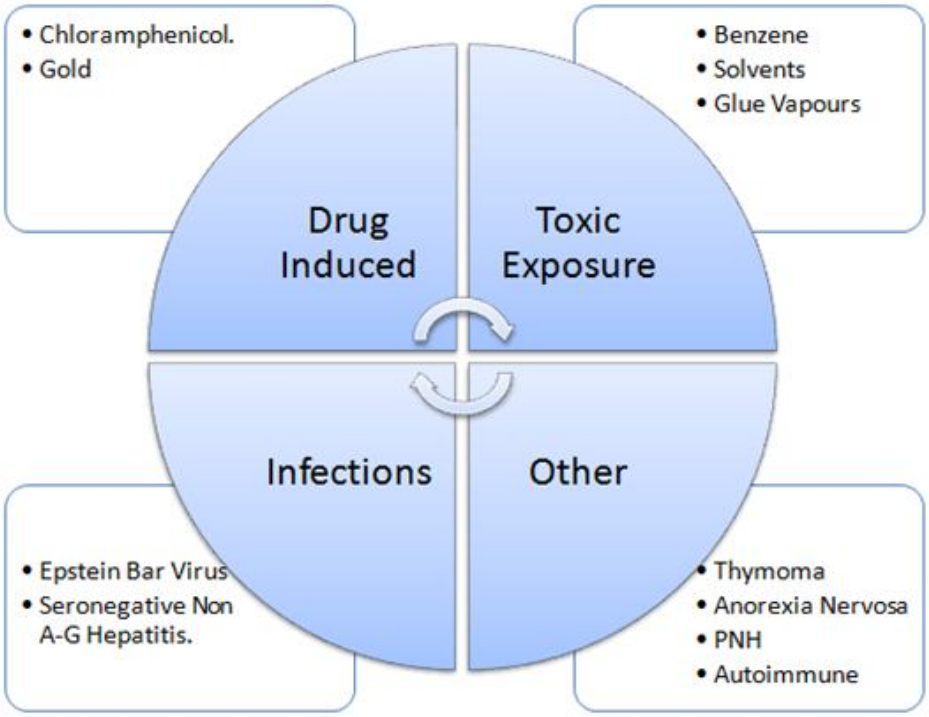

Figure 3. Etiologies of the aplastic anemia [1, 2].

Table 1. Main diagnostic characteristics of aplastic anemia and hypoplastic myelodysplastic syndromes [34]

Criterion	Aplastic anemia	Hypoplastic MDS
Cytopenia	Present	Present
Bone Marrow Cellularity	Aplastic (<10% Cellularity)	Hypocellular
Hematopoiesis		
Erythropoiesis	Present in nests, or "Hot Spots"	Present
Myelopoiesis	Typically decreased	Present
Megakaryopoiesis	Decreased or absent	Present
Dysplasia		
Erythropoiesis	Possible	Possible
Myelopoiesis	Normal Morphology	Possible
Megakaryopoiesis	Normal Morphology	Possible
Blasts	Absent	Variable
CD34+ or CD117+	Nearly Absent	Normal or increased
Marrow Fibrosis	Absent	Possible
Karyotype	Clonal abnormality possible (about 12%)	-7 /del (7q) -5/del(5q)
PNH Clone	Frequent	Unusual
Splenomegaly at Diagnosis	Absent	Possible

Table 2 Diagnostic criteria for AA based on the results of additional tests [1].

Aplastic anemia (AA)	Hematological parameters
Severe aplastic anemia (SAA)	Bone marrow cellularity less than 25% or 25–50% with less than 30% residual hematopoietic cells for two of the three criteria below: neutrophils less than 0.5×10^9 /L; platelets less than 20×10^9/L; reticulocytes less than 20×10^9
Very severe aplastic anemia (VSAA)	Similar to SAA, but neutrophils less than 0.2×10^9
Non-severe aplastic anemia (NSAA)	Patients who do not meet the criteria for SAA and VSAA but having poor cell bone marrow, who have two of three criteria: neutrophils less than 1.5×10^9 /L; platelets less than 100×10^9 /L; hemoglobin concentration less than 10 g/dL

2.2. Classification of aplastic anaemia.

AA has been classified into three groups of moderate, severe and very severe cases as illustrated in table 3. The very severe AA is characterized by absolute neutrophil count less than 200/mm^3, while the moderate is characterized by decreased bone marrow cellularity [33].

Table 3. Classification of aplastic anemia based on severity [33]

Moderate Group	**Severe group**	**Very severe group**
Reduction of bone marrow cellularity	Bone marrow cellularity less than 30% Two of three peripheral blood criteria: -Full neutrophil count -platelet count -reticulocyte count of 500-200 /mm^3 Less than 20.000/mm^3 Less than 40.000/mm^3	absolute neutrophil count less than 200/mm^3
Decrease of at least two of the three hematopoietic lineages not associated with the severity criteria as specified in the right column.		
Depression of at least two of	Involves Patients that fulfill	

the following three hematopoietic lineages	the criteria for severe aplastic anemia but having an absolute neutrophil count of less than 0.2 x 10^9/L.	
Absolute neutrophil count is less than 0.5 x 10^9/L. Patients are those with pancytopenia who do not meet the criteria of disease severity	Depend on Transfusion, with absolute reticulocyte count less than 60 x 10^9/L or platelet count less than 20 x 10^9/L.	

2.3. Management of aplastic anaemia.

Patients with moderate AA are managed using an individualized approach and by considering the symptoms, disease severity, and changes in the degree of cytopenia over time. Close therapeutic monitoring is usually very important, especially when symptoms and transfusion requirements are minimal [45]. Severe aplastic anaemia (SAA) or very severe aplastic anaemia (vSAA) on the other hand, has significant treatment success. It is reported that over 70% could die within one year if not on any treatment [46]. Patients are usually advised not to participate in initial trials of G-CSF or erythropoietin for health safety [47]. There are various treatment options available although, Immunosuppressive Therapy (IST) is still considered the most widely used first line treatment. Diagnosis is based on the severity of aplastic anemia and the age of the patient as shown in Figure 4.

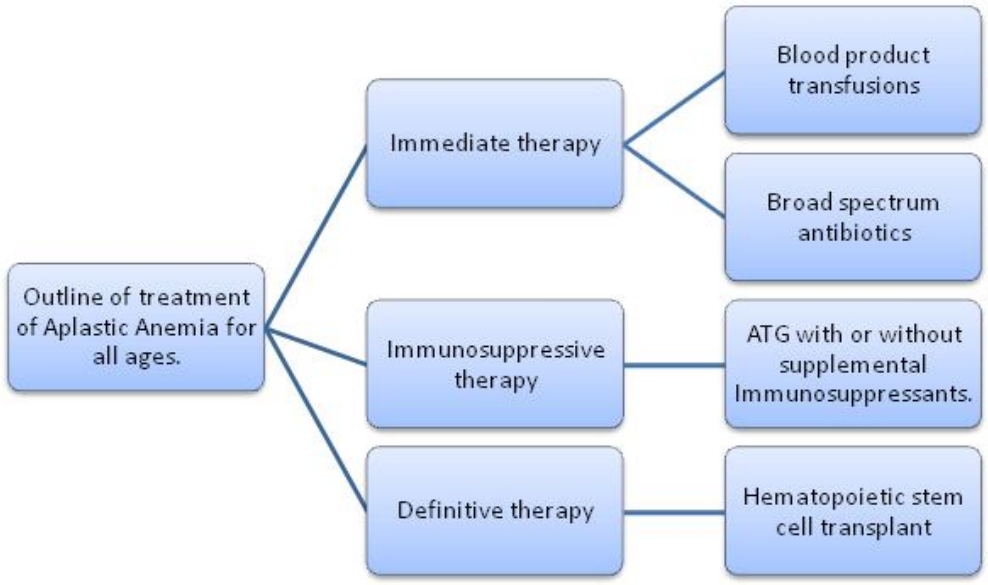

Figure 4. The schematic representation of treatment outline of Aplastic Anemia across all ages [2]

2.4. Common treatment strategies.

The specific aim is to eliminate the symptoms of aplastic anaemia and thrombocytopenia.

A. A method of Packed red blood cell (PRBC) transfusion – There are no reported available specific cutoff of haemoglobin and haematocrit. Transfusion is only advisable when the patient has asymptomatic anaemia, by preference by using leucocyte can reduce and irradiate blood. The excess use of blood products should be encouraged [41].
B. Platelet transfusion is possible when the platelet count is less than 10,000/µl, or when there is any evidence of bleeding.
C. There is need for an institution of a broad-spectrum parenteral antibiotics use [47, 48].

2.5. Immunosuppressive treatment

2.5.1. Anti-thymocyte globulin (ATG)

Immunoglobulin G (IgG) against human antigen-reactive T lymphocytes can cause either the elimination of T lymphocytes in peripheral blood or an alteration in T-lymphocyte function. In aplastic anaemia the IgG may induce complete or partial haematologic signals [49]. It has a half-life of 1.5-12 days; and its prescription can be advised by a clinician for use only for those presented with immunosuppressive therapy and in the case when the patients are advised only to receive the drug in centres equipped and staffed with adequate laboratory and supportive medical platforms [49]. The Anti-thymocyte globulin (ATG) can be administered by a double lumen central line and the platelet count should be less than or equal to 20,000K/µL. Beta-blockers are not advisable for administration before the administering of ATG, in order to avoid the suppression of physiologic responses to anaphylaxis. ATG has adverse effects in patients showing a history of hypersensitivity to antithymocyte globulin, and other equine gamma globulins [42, 50]. An ATG dermatological test can be performed for hypersensitivity to horse serum followed by desensitization if there is reaction to intradermal injection. The treatment with ATG can be stopped where there is evidence of anaphylaxis, unremitting thrombocytopenia, or unremitting leukopaenia [43]. Aplastic anaemia patients may need prophylactic platelet transfusions. Patients are observed and carefully monitored for previously masked reactions when reducing the dose of corticosteroids, and other immunosuppressants [44]. A randomized clinical trial reported by Scheinberg et al., concluded that rabbit ATG was inferior to horse ATG as the initial treatment of SAA, as reported by the haematological response and survival [39].

2.5.2. ATG with cyclosporin (CsA)

A more intensive treatment regimen including ATG and cyclosporine have been shown to provide better results when compared with treatment with ATG alone on patients with SAA [40, 50]. CsA treatment is administered on Day 1. The starting administered dose is usually 10 mg/kg per day (15 mg/kg/day in Pediatric cases). The target minimal therapeutic level ranges between 200 and 400 ng/mL. For high blood pressure patients, treatment is recommended for patient to with an anti-hypertensive like amlodipine and start azithromycin for treatment of gingival hypertrophy [51]. In situation where there is severe renal functioning or creatinine less than 2 mg/mL in patients, temporary cessation of administration of CsA therapy can be done and later reintroduction at lower doses that can be further increased. Recommendation is advised on antimicrobial prophylaxis for *Pneumocystis carinii* with monthly aerosolized pentamidine while

the patient is on therapeutic doses of CsA. While Sulfa drugs are discouraged for administration, alternative regimen with Dapsone or Atovaquone are administered when Pentamidine cannot be used or in very small children [41]. Report indicates that antiviral, antifungal and antibacterial, prophylaxis is seldom administered with standard horse ATG/CsA [41].

2.5.3. Compounds added to ATG+CsA

The addition of therapeutic agents like GCSF, mycophenolate, mofetil, sirolimus danazol, and erythropoietin have been studied in prospective randomized clinical studies with no known difference in response, relapse, clonal evolution or survival [42-46].

2.6. Other agents

Application of a high dose of cyclophosphamide, modified high dose of cyclophosphamide plus cyclosporine, anti-IL- 2 receptor antibody, daclizumab IV can possibly be administered every other week for a total of five doses, and also arsenic trioxide plus cyclosporine [52]. A simple definition of haematological response is not restricted to meeting the blood count criteria for SAA, that is closely linked with transfusion independence and long-term survival. Most of the haematolytic responses (90%) can occur within 3 months after ATG administration [53]. Cyclosporine administration is considered as a common routine practice, however, adequate prospective comparative studies of such a treatment strategy are lacking. Anecdotal and retrospective reports support taper to lower the rate of relapse [54].

2.6.1. Haematopoietic stem cell transplantation (HSCT)

Allogeneic haematopoietic cell transplantation (HCT) is therapeutic, but can be very limited by the availability of a human leukocyte antigens **(**HLA)-matched sibling [54]. Bone marrow is the main source of stem cells in AA, not peripheral blood, unlike other haematological neoplasms [55, 56]. Matched unrelated - donor transplantation is targeted towards patients with failed IST administration, particularly in children and young adults [57].

In patients below 20 years old with SAA or vSAA, with a human leucocyte antigens (HLA)-matched sibling, treatment with allogeneic HCT over treatment with an immunosuppressive regimen is recommended [58]. In patients of 20-50 years age range with SAA or vSAA in a healthy state with a fully HLA-matched sibling donor, patients are recommended to be treated with allogeneic HCT preferred over the treatment with an immunosuppressive regimen. For patients without a matched sibling donor, immunosuppressive treatment is recommended over the use of matched unrelated, mismatched related, or mismatched unrelated HCT [59]. In patients over 50 years of age with SAA or vSAA, the use of immunosuppressive therapy over HCT is recommended due to the fact that there are very high risk of graft-versus- host disease in patients above 45 years [60, 61].

2.6.2. Detection/Diagnosis and survival.

In the absence of AA treatment most patients can have a high mortality rate of about 70% within one year [62]. In most cases, the clinical course varies with complications due to pancytopenia

caused by infections, bleeding, relapse and clonal evolution. On the other hand, increasing intervention with haematopoetic stem cell transplant and an ffective immunosuppressive therapy, survival rates can improve with increases as high as 80% [63].

2.7. The Relationship of Drug Therapy to Aplastic Anemia in clinical settings

2.7.1. Aplastic anaemia Pharmacotherapy

Drug-induced aplastic anemia has remained a serious outcome of modern pharmacotherapy. The incidence of idiosyncratic, drug-induced aplastic anaemia varies based on the genetic diversity and sensitivity linked to the specified drug choice of use [64]. Few studies have elucidated the epidemiology and defined incidence and severity linked to pharmacotherapy and to show that the drugs are the important risk factors known to be associated with aplastic anaemia. Studies have also identified a host range of pharmaceutical products associated with the development of aplastic anaemia. Avoidance of suspected drugs can possibly reduce the incidence of aplastic anaemia and also improve the quality of life of patients [65]. The known associations between aplastic anemia and exposure to carbamazepine with odds of 2.7 has been reported.

Carbamazepine is the most commonly prescribed and administered drug for AA with known association as documented in different case reports [28-30, 66]. Carbamazepine, phenytoin, and phenobarbitone are the first-choice antiepileptic drugs and irrespective of the availability of newer antiepileptic drugs, these drugs are widely used because of their effectiveness and pharmacoeconomic advantages. All the three drugs induce both dose-related toxicity and hypersensitivity, adverse effects on the liver, brain, kidney, gastrointestinal and haemopoietic systems [66]. The risk of aplastic anaemia linked to the use of other antiepileptic drugs like sodium valproate, phenytoin, and felbamate, has been reported in several case reports.

There is a strong association documented in a study in Thailand, to show that the risk of aplastic anaemia increases with administration of a combination of trimethoprim [67]. In African settings, increased use of sulfa-containing drugs is linked with over-the-counter availability of the drugs and self-medication to treat infections. Among all the drugs, chloramphenicol is the most common cause of aplastic anemia and can contributes to 20% to 30% of the total cases [68, 70]. In the United States alone, about 50% of aplastic anaemia cases are known to be caused by chloramphenicol since 1952 [71]. Globally, despite the effectiveness of chloramphenicol, low cost, and few numbers of side effects, its use has been limited for the treatment of AA. However, its withdrawal from the markets on the contrary has not shown any reduction in the incidence of aplastic anaemia [71]. Based on this controversial discussion, Surapol Issaragrisil et al proposed that more research be done on the risk of chloramphenicol use among AA patients [70-73].

2.7.2. Mebendazole

Mebendazole, is an anthelmintic bioactive molecule of specific interest due to the fact that is it very closely associated with aplastic anaemia and similar association has been confirmed globally [72]. In Pakistan for example, herbal products or home herbal medicines uses are very common as the community believe that they are effective in the treatment of different ailments.

These herbal remedies are formulated to well-known or unknown drugs or bioactive moleculaes or phytomedicines [46, 73] that might be link with aplastic anaemia [74].

2.7.3. **Trimethoprim-sulfamethoxazole (TMP-SMX)**

TMP-SMX is a bacteriostatic antimicrobial drug used for the treatment of a wide range of bacterial infections under the trade name Bactrim, and has been linked to many reported skins and haematologic adverse effects [75]. Due to the availability and low cost, TMP-SMX is one of the medications commonly used for the treatment of skin and soft tissue in patients with methicillin-resistant *Staphylococcus aureus* (MRSA) infection [60]. One of the rare haematologic manifestations of TMP-SMX is pancytopenia, which causes a reduction in all cell lines [76]. There is a well-documented link between this drug and other related drugs such as carbamazepine, thiazides, and mebendazole. The risk of the idiosyncratic reaction of Bactrim from one patient to another. It is even recommended that physicians understand the possible mechanism of drug-related aplastic anaemia so as to revise and improved on their significantly the prescription habits [77]. There is an urgent need for the development of capacity building platform for clinicians to enhance continuous surveillance of the safety and efficacy of the use of pharmaceutical products [78].

2.8. Eltrombopag: administration in patients with severe aplastic anaemia

Eltrombopag (Promacta®) is an active oral thrombopoietin receptor agonist that has been approved in the US for the treatment of patients with severe aplastic anaemia showing an insufficient response to immunosuppressive treatment [68]. Eltrombopag is noncompetitive with thrombopoietin and bind to a different site on the receptor, thereby producing additive effects. It can stimulate haematopoietic stem cells and promotes haematopoietic recovery in patients with aplastic bone marrow conditions. Eltrombopag also has the capacity to enhance platelet counts, red blood cell and neutrophil counts [67, 69]. In the case of patients with severe aplastic anaemia refractory to prior immunosuppressive therapy, oral eltrombopag at dosages less than 50 mg administered once daily for 12-16 weeks are known to produce a haematological response in at least one cell lineage in 40 % of patients. Trilineage responses has been achieved in nearly one-half of the responders during extended treatment. In robust responders, stable haematological counts were maintained after eltrombopag discontinuation. Eltrombopag was shown to be well tolerated, with increased in liver transaminases as the only dose-limiting toxicity [66, 67]. Clonal cytogenetic abnormalities have been observed in 19 % of patients and dysplasia in 5 % of patients.

3.0. NATURAL HERBAL PRODUCTS IN APLASTIC ANAEMIA MANAGEMENT.

Most patients in West and Central Africa subregions where sickle cell anemia (SCA) is endemic have in the past three decades been treated with natural herbal products known to be safe, easily accessible and environmentally friendly. This practice is still in progress in the rural enclaved communities where the population have limited access to health facilities and therefore depend on category one traditional medicine use. Studies have also shown that some of these herbs have

promising potential for the optimization of the treatments of patients [76, 80]. Further studies have revealed that during the last 2-3 decades, much progress has been made in several aspects of anaemia pharmacology, especially the approval of the molecule hydroxyurea [78]. As for anaemia herbal use for treatment, much has been revealed that antisickling herbs are common in West Africa and that the most promising are still to be discovered. Three reported new antisickling herbs *Entandrophragma utile*, *Chenopodium ambrosioides*, and *Petiveria alliacea*, have been widely used in resource limited countries [76, 77], and has motivated interest into more innovated traditional medicine research in tropical herbs for AA treatment. The discovery of more effective herbal products for treatment of aplastic anaemia could be more promising to compensate for the time and effort in research.

There are many published information on the effects of different herbal extracts and their isolated bioactive compounds on human and other mammalian stem cells isolated from different plant sources. For instance, studies done in 2016 focused on osteogenic, anti-adipogenic, neurogenic, endothelial/vascular genesis, angiogenesis and proliferative effects of herbal extracts on human mesenchymal stem cells (hMSCs), that was later confirmed by RNA gene expression studies [80]. Dried root of Korean herb *Dipsacus asper* for a long time had been used in Korean traditional medicine for the treatment of bone fracture and the crude extract, and an isolated compound from the herb hedraganin-3-O-(2-O-acetyl)-α-L-arabinopyranoside demonstrated the osteogenic differentiation ability on bone marrow-derived hMSCs via the upregulation of bone-specific proteins and alkaline phosphatase activity [79]. *Aloe emodin*, also present in Aloe latex, showed anti- adipogenic activity on hMSCs by down regulation of the expression levels of mRNAs resistin, adiponectin, aP(2, lipoprotein lipase, PPARγ and tumour necrosis factor-α) involved in adipogenic pathways [77]. The treatment of adipose-derived hMSCs with dried root extract of *Angelica sinensis*, as a medicinal product commonly used in traditional Chinese medicine, resulted in a very differentiation of neural-like cells than a commonly used neural inducer, butylated hydroxyanisole [80]. The neuroprotective potential of the same extract showed a decreased induced neurotoxicity in cultured cortical neurons, thereby increasing the extract's value as a potential candidate for treating neurodegenerative disorders [77, 80].

4.0. PHARMACOECONOMICS OF THERAPEUTIC INTERVENTION FOR APLASTIC ANEMIA

Currently, the best treatment consideration for aplastic anaemia is the bone marrow transplant also known as stem cell transplant, and widely considered the only known and guaranteed cure for aplastic anemia [83]. Bone marrow transplants can replace damaged stem cells with healthy ones. Immunosuppressive therapy with antithymocyte globulin (ATG) and cyclosporine is the standard treatment for patients with severe aplastic anemia who do not have a human leukocyte antigen-matched related donor; that can result to a response rate of 60 to 70% [75].

Administration of ATG is easy, as it is injected into a 1000 ml bag of normal saline, and attached to the cell line or Hickman line, then allowed to run for around 12 hours a day. The treatment duration is normally estimated for about 4 days. Before having the first full bag, it is advisable

for a small dose to be given over an hour as some patients may have allergic responses [66]. Therefore, idiopathic aplastic anaemia is a diagnosis of exclusion and the clinical severity is classified based on the peripheral blood counts and the results of bone marrow examinations. The signs and symptoms of AA are mainly those associated with pancytopenia. Immunosuppressive therapy (IST) has shown low response, toxicity, and risk of transformation [67]. In a Phase I and II clinical trial, the addition of eltrombopag to first-line IST indicated an increased response rates relative to an IST-only historical cohort. A model has been developed to estimate the budget impact of treating SAA with eltrombopag-based therapy from a US private healthcare system perspective [72].

A simulated cohort of newly diagnosed SAA patients based on the total US population received 6 months of IST with or without eltrombopag and were monitored for about a year, with mutually exclusive patient cohorts entering in years 1, 2, and 3. The model assessed the budget impact of first-year treatment for each cohort without considering subsequent years [56, 82]. At 6 months, responders in either arm received maintenance therapy made of low-dose cyclosporine, and non-responders received 6 months of second-line eltrombopag monotherapy [68].

Costs for consideration included first-line, maintenance, and second-line therapy, administration, routine care, mortality, and adverse events (AEs) [65]. All cost data in 2018, were in US dollars. The annual incidence of aplastic anemia was 0.000234%, with 83.8% of cases assumed to be SAA [69, 81]. Based on trial data, 94% of patients receiving eltrombopag and IST responded versus 66% of patients receiving IST, with a 0.3% reduction in the annual risk of mortality for the eltrombopag plus IST group. The use of first-line eltrombopag in a model SAA population based on the total US population increased overall costs by $50 million over 3 years [82] First-line drug costs accounted for an increase of $69 million, while improved response produced $19 million in secondary therapy cost savings [84, 85]. Sensitivity analyses confirmed the robustness of the analysis. So far, there is a gap of information on the pharmacoeconomic implication of SAA therapy in limited income resource countries, due to little information on the research interest, and limited access to research data that has not been published.

5.0. APLASTIC ANEMIA IN PAEDIATRICS.

Aplastic anemia is a serious condition in which the bone marrow does not make enough new blood cells. With low blood cells, a child with AA has reduced oxygen sent to the organs, tissues, and cells from too few red blood cells [81]. Children also have an increased risk of infection due to too few white blood cells, and an increased risk of severe bleeding problems from too few platelets. AA in children may result from many causes [86], sometimes the causes are not known. AA may develop at some point during the development stage of the child, or it may be passed down from parent to child.

5.1. The acquired causes of aplastic anaemia in children.

Those causes that can lead to the production of AA in children include;

Viral infection. These include hepatitis or liver infection, and many different viral diseases, such as parvovirus B19, or human immunodeficiency virus (HIV), Epstein-Barr virus (EBV), and cytomegalovirus (CMV), [88].

Neoplasm-Cancer. Some cancers affect the bone marrow. The plasma cells contain white blood cell in the bone marrow. In AA condition, a group of plasma cells becomes cancerous and multiplies. The disease can thus damage the bones, immune system, kidneys and reduces red blood cell count. AA symptoms may not experience or may be non-specific, such as loss of appetite, bone pain and fever. Treatments include medication, chemotherapy, corticosteroids, radiation or a stem-cell transplant.

Autoimmune disease. When your immune system attacks your own body, you are said to have acquire an autoimmune disease. Other autoimmune diseases linked to aplastic anaemia include rheumatoid arthritis and lupus. Acquired aplastic anemia can have an onset at any time in life. However more than 75 out of 100 cases of acquired aplastic anemia are idiopathic.

Drugs. Exposure to radiation (radiation sickness) Chemotherapy. Environmental toxins (insecticides, benzene, nitrogen mustards) Many different medications, including chloramphenicol (Chloromycetin), phenylbutazone (Butazolidin), sulfonamides (Gantanol and others), anticonvulsants, cimetidine (Tagamet) and others.are in prescription or not known.

Toxins. These include heavy metals, pesticides, and benzene. Toxic chemicals, such as those used in pesticides and insecticides, benzene, and substances in gasoline, have been linked to aplastic anaemia. This type of anaemia may improve when repeated exposure is reduced drugs

Radiation therapy and chemotherapy. These are drugs developed to treat cancer. Aplastic anaemia is not cancer, however, it can be caused by common cancer treatments such as radiation and chemotherapy

5.2. The symptoms and diagnostics of aplastic anemia in children.

The most common symptoms of aplastic anaemia includes;

Low levels of red blood cells that can cause irregular heartbeat, pale skin, chest pain, and enlarged heart. Low concentration levels of white blood cells can provoke fevers, mouth sores, and infections. Low levels of platelets production can cause easy bruising, heavy bleeding with menstrual periods, nosebleeds, bleeding gums, blood in the stool, and [85]. Other symptoms may include nausea and skin rashes, dermatological complications. The symptoms of aplastic anemia may be expressed like other blood disorders or medical problems and therefore there is a need to check with medical experts for a timely and accurate diagnosis.

5.3. Diagnostic of aplastic anaemia in children

Children healthcare provider usually refer a child to a hematologist who specialises in blood disorders, along with a complete medical history and physical examination of the chil. The tests for aplastic anemia may include [81]:

Haemoglobin and haematocrit. This blood test measures the amount of haemoglobin, the part of red blood cells that carry oxygen, and the iron rich protein in the red blood cells in the blood. Often, the first line test used to diagnose aplastic anemia is a complete blood count (CBC). The CBC measures many parts of the blood. This test checks hemoglobin and hematocrit (hee-MAT-oh-crit) levels. A complete blood count checks determines the red and white blood cells, blood clotting cells (platelets), and sometimes, young red blood cells (reticulocytes).
Peripheral smear. A small sample of blood is examined under a microscope and the blood cells are checked to evaluate their normality.
Bone marrow aspiration or biopsy. A doctor uses a needle to remove a small sample of bone marrow from a large bone in the body, such as the hipbone. The sample is examined under a microscope to rule out other blood-related diseases. In AA, bone marrow contains fewer blood cells than normal. This process involves taking a small amount of bone marrow fluid called aspiration or solid bone marrow tissue called a core biopsy. The hip bone is often used for the biopsy specimen. The fluid and tissue are examined for the number, size, and maturity of blood cells or abnormal cells [68, 82].

5.4. The treatment of aplastic anaemia in children.

The treatment of AA for paediatircs depend on a child's symptoms, age, general health, and the severity of the condition reported. The treatment for aplastic anemia also depends on the cause and for mild aplastic anaemia, treatment may not be required or may need blood transfusions, platelet transfusions, antibiotics, hormones or other drugs prescribed by a clinician to stimulate the bone marrow to produce cells, immunosuppressive medicine, and stem cell transplant [65, 82].

Treatment that may lead to some complications.

With an appropriate treatment, the risk of complications may reduce in aplastic anaemia. However, the complications may include treatment-related side effects, problems with growth and development, cancers, heart failure, continuous bleeding, and in some cases very severe infections [73, 84].

5.5. Primary parental intervention in children with aplastic anemia

There is a need to work with a child's healthcare provider to develop a treatment plan, in order to normalize children's life as possible. Efforts have to be made to focus on other children in the family [87]. Parents are advised to work closely with their children's schools to make sure they get what is needed to support AA patients. A child suffering from AA may also qualify for special rehabilitation programs in some well-developed countries. For AA kids who feel different or alone, it is necessary to find a support group for them within the community. Kids suffering from AA are advised to reduce or avoid activities that increase the chances of bleeding. Such activities include things like avoiding contacts with sick individuals in order to reduce infection transmission, to avoid eating uncooked foods, contact sports (for example, football, hockey, skiing, or rollerblading) and traveling to high altitudes. Children with a low red blood cell count will have increased fatigue and need the for oxygen at high altitudes) [88].

Conclusion

The treatment of severe aplastic anemia either by allogeneic stem cell transplantation or immunosuppression, has shown significant progress over the years, with records of long-term survival of more than 75% of patients reported. A multidisciplinary approach is necessary for the management of important result outcomes and to develop promising treatment options. Research interest towards seeking expert advice on the diagnosis and management of patients where diagnosis is difficult is necessary, or when an inherited bone marrow failure syndrome is being considered. More motivation should be given for participation in clinical studies for drug testing for AA therapy. It is necessary for clinicians to be sensitized on the possibility of drug-related aplastic anaemia and importance on drug adherence, and review their prescription regimen regularly. There is also an urgent need for capacity building initiative platform for clinicians and continuous surveillance of the safety and efficacy of the pharmaceutical products which are used in their clinical practice for aplastic anaemia interventions. There is a high recommendation for the development of a formulation guidelines of new legislation for drug safety and national guidelines for pharmacovigilance in AA drug adverse effects and safety pharmacology. A general sensitization and advocacy is needed for the risk of auto medication in the population on target antibiotics use that are of potential predisposition risk and adverse effect to aplastic anaemia patients

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