Review Article

The Scope of Aplastic anaemia: Etiology, Pathophysiology, pharmacotherapy and Pharmacoeconomic impact in clinical patient management.

ABSTRACT

Aplastic anaemia (AA) is a rare but sometimes ~~a~~ life-threatening health condition that accounts for one of the causes of bone marrow failure, and could trigger bone marrow hypoplasia or aplasia. AA may lead not only to anaemia but also can cause pancytopenia. The most immediate cause of AA is an autoimmune reaction of T lymphocytes against hematopoietic stem cells or, to a lesser ~~extend~~ extent in some cases, a congenital defect or acquired damage to these cells leading to inhibition of their cell division and eventual differentiation. AA can quickly develop within a few days or slowly for several weeks or months. The signs and symptoms are ~~associated~~ due to anaemia, neutropenia, and thrombocytopenia. The treatment strategies of patients with AA have improved progressively with time due to better outcomes of both family and unrelated donor haematopoietic stem cell transplantations (HSCTs) as well as the revised protocol of immunosuppressive therapy (IST). The treatment ~~method~~ options depend principally on three factors such as the severity of AA, the age of the patient and matched siblings' donor. All patients diagnosed with AA require appropriate supportive treatment adapted to the current clinical settings. Supportive treatment is important both before, during and after invasive causal treatment, ~~that~~ which principally involves the transfusion of leukocyte-depleted blood components, the use of anti-infectious prophylaxis or treatment of infections. In many situations, ~~AA,~~ supportive therapy is the only therapeutic option, especially for elderly patients with comorbidities. This paper's ~~has as~~ objective is to give an insight into the scope and concepts of AA, etiology, pathophysiology, therapeutic options, treatment complications and pharmacoeconomics of treatment and management.

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

1. INTRODUCTION

Aplastic anaemia is a rare bone marrow disorder characterized by pancytopenia [1]. It could be congenital in nature, idiopathic but in less cases are caused by some drugs, chemicals and infections [2]. It is diagnosed with hypocellular bone marrow, and the most suitable treatment is an allogenic haematopoetic stem cell transplant. However, supportive care with transfusion and immunosuppressive therapy could enhance symptomatic relief and ~~enhanced~~ quality of life for most patients [1-3]. AA is considered as an acquired or congenital bone marrow failure in the production of all cell lines, without the presence of cancerous infiltrates and fibrosis, that can lead to pancytopenia. It is a disease that occurs rarely, however, it has serious challenges in prognosis in the absence of adequate treatment [4]. With the development of effective therapies

so far, the prognosis has changed significantly, mostly in pediatric cases with severe pancytopenia, but results of specialist treatment are conditioned by appropriate supportive care. The incidence in Europe and North America is estimated at 2–3 cases/million/year and in Asia, 5–6 cases/million/year [4, 5]. AA can occur at any age, but most cases fall between 15–25 years and over 60 years, without gender or race variations as its etiology is well understood [5, 6].

The designation "aplastic anemia" is a misnomer, due to the fact that the disorder is characterized by pancytopenia (A condition in which there is a lower-than-normal number of red and white blood cells and platelets in the blood) [7]. Pancytopenia occurs 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 can likely lead to a reduction in the pluripotent stem cell number below a critical threshold mass. This is due to a conflict between self-renewal versus differentiation that can result ~~to~~ in an ultimate stem cell or bone marrow failure as shown in figure 1. There is a complex balance between stem cell differentiation and regeneration in the etiology of aplastic anemia [2, 8]. In most cases, AA is considered idiopathic in nature, although it can be associated with cytotoxic drugs (chloramphenicol, gold) [9], radiation, toxic chemicals (like Benzene, solvents and glue vapours), viral infections (Epstein-Barr Virus Infection, Seronegative Non A-G hepatitis) [11], immune-related disorders (Eosinophilic fasciitis, SLE, Graft versus host disease) [10], 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 is illustrated in figure 1.

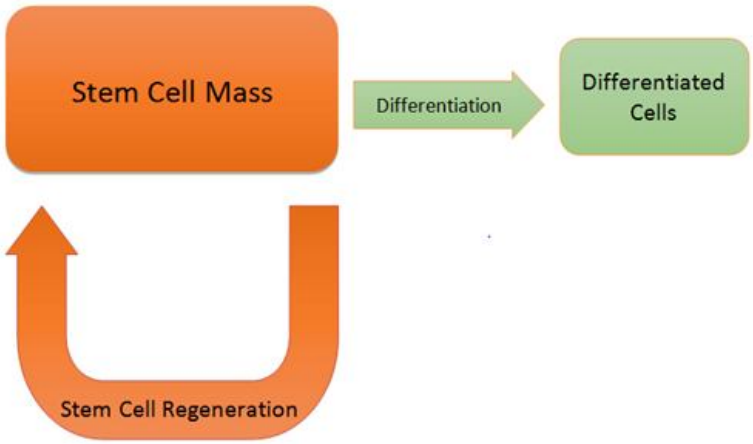

Figure 1 Showing the complex balance between stem cell differentiation and regeneration in the etiology of aplastic anemia [2]].

1.2. Drug induced Aplastic anaemia

Most patients exposed to cytotoxic drugs do not develop AA, and the exact mechanism for the idiosyncratic reaction is not well understood. The P-glycoprotein, Multi-Drug Resistance Gene, (MDR-1) gene product, and the multidrug resistance-associated protein (MDR-AP), are energy-dependent transmembrane efflux pumps for a variety of lipophilic drugs [2, 11]. They are responsible for keeping the drugs out of the cells as illustrated in figure 2 which shows the mechanism of drug efflux from the cell [12]. An overexpression of the P-glycoprotein ~~renders the~~ confer on cancer cells a multidrug resistance phenotype ~~to cancer cells~~, whereas an under-expression in normal cells allows cytoplasmic accumulation of drugs and enhances their toxic effects [13-14].

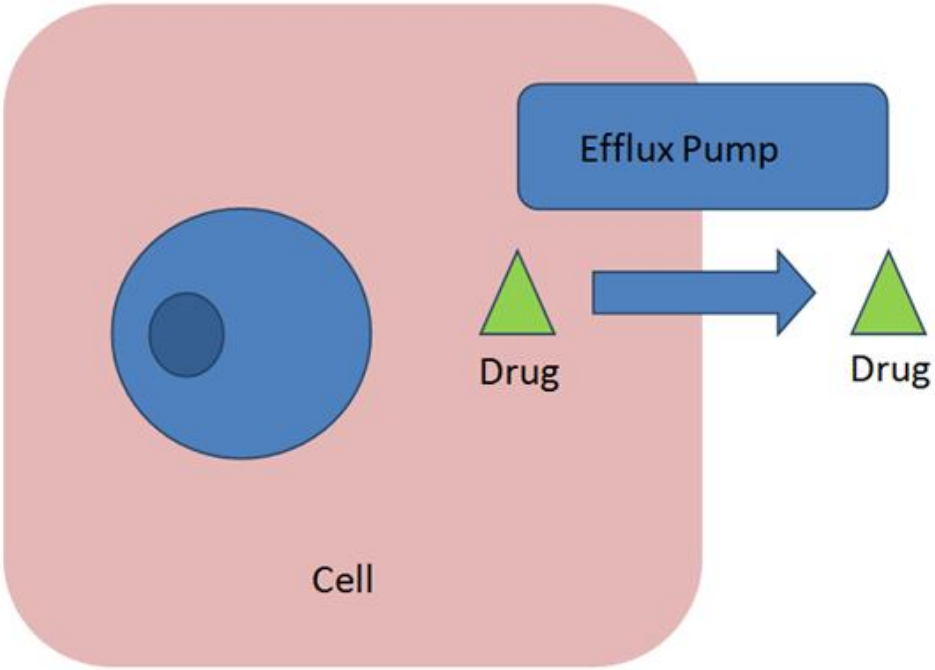

Figure 2: The mechanism of drug efflux from the cell [2].

1.3. Aplastic Anaemia caused by Viral infections

Certain viruses are implicated in causing AA as indicated.

1.3.1. Parvovirus B19

This virus generally attacks pro-erythroblasts and causes transient red cell aplasia, that has been reported in patients with chronic haemolytic anaemia [12]. However, pancytopenia is generally seen in patients with immune-compromised situations [3, 14]. Human parvovirus B19 has been associated with a broad spectrum of diseases including *Erythema infectiosum* (EI) in children. One of the most common and serious complications of parvovirus B19 infection is transient aplastic crises in patients with chronic haemolytic anaemia such as sickle cell disease and

hereditary spherocytosis [15]. Pure red cell aplasia may also develop with a persistent infection of parvovirus B19 in immunocompromised individuals [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 children with SAA has been done to explore the relation between parvovirus B19 infection and aplastic anaemia [17, 18].

1.3.2. Hepatitis virus and human immunodeficiency virus (HIV)

These viruses may involve T cell activation with the release of cytokines or activation of ~~a~~ cytotoxic T cell clones which recognize similar target antigens on both liver and bone marrow cells [19, 20]. Hepatitis-associated aplastic anaemia (HAAA) is a well-recognized and distinct variant of the clinical syndrome, acquired aplastic anaemia, in which an acute attack of hepatitis leads to ~~the~~ marrow failure and pancytopenia [20-21]. HAAA was first reported in two cases by Lorenz and Quaiser in 1955 [21] and the number of the cases increased ~~up to a value of~~ to 200 by the year 1975 [22]. However, this syndrome has been reported in 2-5% cases in developed countries and 4-10% in areas ~~of more~~ with increased prevalence ~~to~~ of hepatitis and Human Immunodeficiency Viruses (HIV), in the Far East Asia [22], ~~of~~ and low socioeconomic status [19]. HAAA is not considered relative to age, sex and severity of hepatitis. Predominantly, it has been found in children, adolescent boys, and in young aged men [23].

1.3.3. Aplastic Anaemia and viral hepatitis

The pathophysiology is immune-mediated in most cases, and activated T1 lymphocytes have been identified as effector cells. The disease can be 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 most often affects young males who show severe pancytopenia two to three months after an episode of acute hepatitis [24]. The clinical course of hepatitis is more frequently benign in nature. ~~The~~ Bone marrow failure can be explosive and severe and it is usually fatal if untreated, and no correlations have been reported between the severity of hepatitis and AA [25]. The characteristics and response to immunotherapy indicate a central role ~~for~~ of immune mechanism in the pathogenesis of HAA.

The initial target organ of the immune response is the liver as suggested by the time interval between hepatitis and ~~the~~ failure [26]. Liver histology is characterized by T cells infiltrating the parenchyma as reported in acute hepatitis. Recently in HAA, it has been demonstrated intrahepatic 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 anemia [27, 28].

1.4. Auto-immune signature in aplastic anaemia

Acquired idiopathic aplastic anemia (IAA) is a rare haematologic disorder characterized by the failure of haematopoiesis secondary to an immune-mediated damage ~~of~~ to the bone marrow. IAA is naturally considered 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 by using a combined deep sequencing and flow cytometry approach [30]. It is reported that clonally expanded T-cell populations are frequently detectable even within the effector memory compartment, and that they tend to correlate with disease activity. Therefore, the characterization of the T-cell receptor (TCR) repertoire by high-resolution techniques may play a role in confirming the diagnosis of immune-mediated IAA and ~~to monitor~~ monitoring of affected patients during their disease evolution [31].

There is widespread clinical and experimental evidence to support the autoimmune pathophysiology of IAA [31]. The most striking 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]. Almost four decades of investigations have shown a number of experimental data corroborating the hypothesis of an immune-mediated pathophysiology. Increased circulating activated T cells was described in IAA patients in the ‘80s [26, 33]. These T cells may suppress haematopoiesis through the secretion of different inflammatory cytokines [34], and/or *via* cell-mediated direct killing. Among the different inhibitory cytokines, interferon-γ (IFN-γ) plays a major role in suppressing human hematopoietic stem cells (HSC) *in vivo*, as suggested by *in vitro* inhibition of cell cycle progression and induction of apoptosis of haematopoietic progenitors [26, 35]. Currently, it has been suggested that IFN-γ may also exert its inhibitory effect on HSC impairing the homeostatic survival signal delivered by thrombopoietin through its cognate receptor c-MPL [27, 36]. This inhibitory environment is generated by immune cells, and mostly by T cells that become activated and proliferate in response to an antigen-driven stimulation. While the search for these putative antigens has remained 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, under the influence of the transcription factor T-bet and/or cytokines such as TNF-alpha and various interleukins [27, 28]. Unregulated 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 results in a haematopoietic inhibitory response. This inhibition may be mediated by IFN-gamma or the cytokine cascade released by IFN-gamma that could result in apoptotic death of haematopoietic stem cells in the bone marrow [28-30].

2.0. CLINICAL MANIFESTATIONS

Patients with AA are usually well, prior to the diagnosis. AA occasionally comes to medical attention because of fatigue and other symptoms associated with progressive anaemia. More common presentations include recurrent infections due to profound neutropenia or mucosal haemorrhage due to thrombocytopenia [31-35]. Infections are typically bacterial, however, invasive fungal infection is a common cause of death; especially in subjects with prolonged and severe neutropenia [37, 38]. Increased menstrual flow is also a common complaint in premenopausal women. AA can have no symptoms but when present, signs and symptoms can include: Fatigue, Shortness of breath, Rapid or irregular heart rate, Pale skin, Frequent or prolonged infections, Unexplained or 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 it can become chronic [39, 40]. It can be severe and even fatal [41].

2.1. Diagnosis

Patients with AA, have clinical findings consistent 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 measurement of red cell membrane or neutrophil CD59 by flow cytometry, and cytogenetics are indicated [34, 43]. Diagnosis of AA is established by demonstration of pancytopenia and hypocellular bone marrow. An important differential diagnosis is ~~of~~ hypoplastic myelodysplastic syndromes (MDS), which should be kept in mind 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

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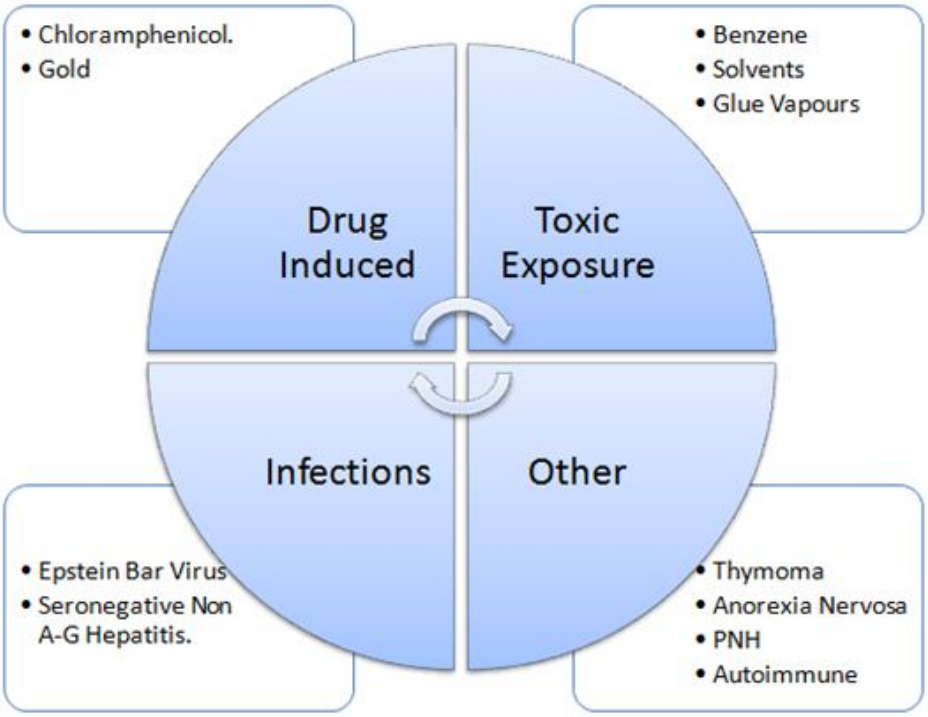

Figure 3. Etiologies of the aplastic anemia [1, 2].

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 <25% or 25–50% with less than 30% residual hematopoietic cells two of the three criteria below: neutrophils $<0.5\times10^9$ /L; platelets $<20\times10^9$/L; reticulocytes $<20\times10^9$ /L
Very severe aplastic anemia (VSAA)	Similar to SAA, but neutrophils $<0.2\times10^9$ /L
Non-severe aplastic anemia (NSAA)	Patients who do not meet the criteria for SAA and VSAA but with poor cell bone marrow, who have two of three criteria: neutrophils <1.5×109 /L; platelets <100×109 /L; hemoglobin concentration <10 g/dL

2.2. Classification of aplastic anaemia.

AA are well classified into moderate, severe and very severe as illustrated in table 3. The very severe AA is characterized by absolute neutrophil count < 200/mm^3, while the moderate is characterized by decreased bone marrow cellularity [33].

Table 3. Classification of aplastic anemia by severity [33]

Moderate	**Severe**	**Very severe**
Decreased bone marrow cellularity	Bone marrow cellularity <30% Two of three peripheral blood criteria: -absolute neutrophil count -platelet count -reticulocyte count 500- 200 /mm^3 <20.000/mm^3 <40.000/mm^3	absolute neutrophil count <200/mm^3
Depression of at least two of the three hematopoietic lineages not fulfilling the		

severity criteria as specified in the right column.		
Depression of at least two of the following three hematopoietic lineages	Patients that fulfill criteria for severe aplastic anemia but with an absolute neutrophil count of <0.2 x 10^9/L.	
Absolute neutrophil count <0.5 x 10^9/L. Patients with pancytopenia who do not meet the criteria of severe disease	Transfusion dependence, with absolute reticulocyte count <60 x 10^9/L or platelet count <20 x 10^9/L.	

2.3. Management of aplastic anaemia.

Patients with moderate AA are managed with an individualized approach considering the symptoms, disease severity, and changes in the degree of cytopenia over time. Close monitoring is often appropriate, especially when symptoms and transfusion requirements are minimal [45]. Severe aplastic anaemia (SAA) or very severe aplastic anaemia (vSAA) on the other hand, are successfully treated, over 70% will die within one year [46]. Patients are advised not to be subjected to initial trials of G-CSF or erythropoietin [47]. There are various treatment options available although, Immunosuppressive Therapy (IST) remains the most commonly used first line of therapy. Prognosis depends on the severity of aplastic anemia and the age of the patient as illustrated in Figure 4.

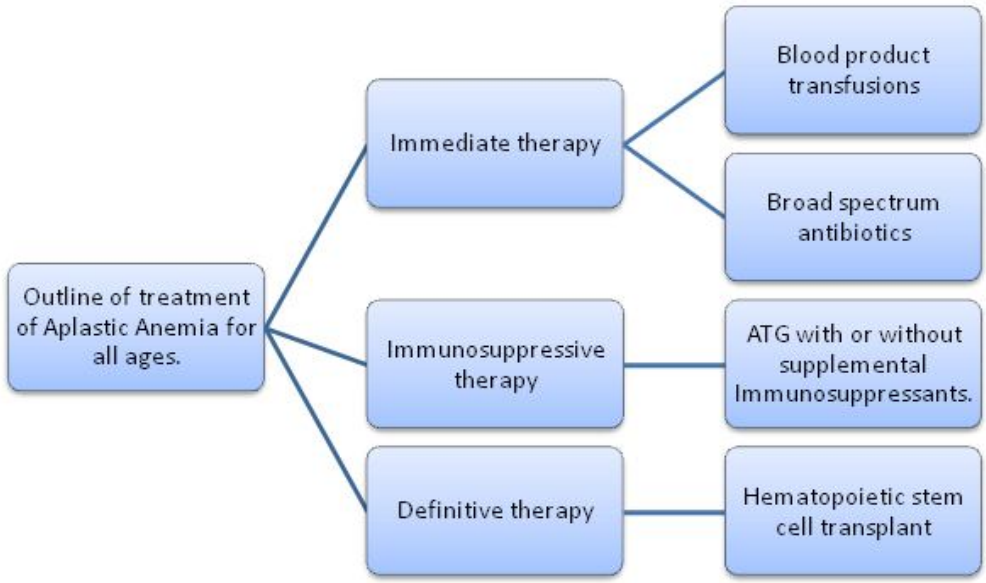

Figure 4. The Outline of treatment of Aplastic Anemia for all ages [2].

2.4. Immediate treatment measures

The immediate aim is to eliminate symptoms of anaemia and thrombocytopenia.

A. PRBC transfusion - no specific cutoff of haemoglobin and haematocrit is available. It is advised to transfuse only if the patient is symptomatic from anaemia, preferably use leucocyte reduced and irradiated blood. Overuse of blood products should be avoided [41].
B. Platelet transfusion if the platelet count is less than 10,000/µl, or evidence of bleeding.
C. Broad spectrum parenteral antibiotics should be instituted [47, 48].

2.5. Immunosuppressive therapy

2.5.1. Anti-thymocyte globulin (ATG)

Immunoglobulin G (IgG) against human antigen-reactive T lymphocytes (equine-derived) causes either elimination of T lymphocytes in peripheral blood or alteration in T-lymphocyte function. In aplastic anaemia these IgG may induce complete or partial haematologic response [49]. It has a half-life of 1.5-12 days; its prescription is advised by physicians only for those experienced in immunosuppressive therapy and patients are advised only to receive the drug in facilities equipped and staffed with adequate laboratory and supportive medical resources [49]. It can be administered by a double lumen central line and platelet count should be ≥20,000K/µL. Beta-blockers should be held before ATG administration to avoid suppressing physiologic responses to anaphylaxis. It is contraindicated in patients with a history of hypersensitivity to antithymocyte globulin, and other equine gamma globulins [42, 50]. An ATG skin test can be performed for hypersensitivity to horse serum followed by desensitization if reacting to intradermal injection. The treatment should be discontinued if there is evidence of anaphylaxis, unremitting thrombocytopenia, or unremitting leukopaenia [43]. Aplastic anaemia patients may need prophylactic platelet transfusions. Patients should be observed carefully for previously masked reactions when reducing the dose of corticosteroids, and other immunosuppressants [44]. A randomized trial by Scheinberg et al., concluded that rabbit ATG was inferior to horse ATG as the initial treatment of SAA, as was reported by the haematological response and survival [39].

2.5.2. ATG with cyclosporin (CsA)

A more intensive regimen including ATG and cyclosporine appears to provide superior results compared with treatment with ATG alone in patients with SAA [40, 50]. CsA administration is initiated on Day 1. Starting dose is 10 mg/kg per day (15 mg/kg/day in children). Target trough level is between 200 and 400 ng/mL. For high blood pressure, it is advised to start anti-hypertensives like amlodipine and start azithromycin for bothersome gingival hypertrophy [51]. If renal functioning worsens or creatinine ≥ 2 mg/mL, temporary cessation of CsA therapy and later reintroduction at lower doses with further increases as tolerated is recommended. Antimicrobial prophylaxis for *Pneumocystis carinii* with monthly aerosolized pentamidine while the patient is on therapeutic doses of CsA is recommended. Sulfa drugs are avoided, alternative regimen with Dapsone or Atovaquone are used when Pentamidine cannot be used or in very small children [41]. Antibacterial, antiviral and antifungal prophylaxis is not routinely administered with standard horse ATG/CsA [41].

2.5.3. Agents added to ATG+CsA

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

2.6. Other agents

High dose cyclophosphamide, modified high dose cyclophosphamide plus cyclosporine, anti-IL-2 receptor antibody, daclizumab IV can be administered every other week for a total of five doses, arsenic trioxide plus cyclosporine [52]. Simple definition of haematological response is no longer limited to meeting blood count criteria for SAA, which closely correlates with transfusion independence and long-term survival. The majority (90%) of the haematological response occurs within 3 months after ATG [53]. Cyclosporine taper is a common practice but adequate prospective comparative studies of such a strategy are lacking. Anecdotal and retrospective reports support taper to decrease the rate of relapse [54].

2.6.1. Haematopoietic stem cell transplantation (HSCT)

Allogeneic haematopoietic cell transplantation (HCT) is curative, but is limited by the availability of an HLA-matched sibling [54]. Bone Marrow is the preferred source of stem cells in AA, not peripheral blood, unlike other haematological neoplasms [55, 56]. Matched unrelated - donor transplantation should be reserved for patients for whom an initial course of IST has failed especially in children and young adults [57].

In patients under the age of 20 with SAA or vSAA, with an HLA- matched sibling, treatment with allogeneic HCT over treatment with an immunosuppressive regimen is recommended [58]. In patients 20-50 years of age with SAA or vSAA in otherwise excellent health with a fully HLA-matched sibling donor, it is recommended to treat with allogeneic HCT over treatment with an immunosuppressive regimen. For those individuals without a matched sibling donor, immunosuppressive therapy is recommended over the use of matched unrelated, mismatched related, or mismatched unrelated HCT [59]. In patients over 50 years with SAA or vSAA, the use of immunosuppressive therapy over HCT is suggested because of the very high risk of graft-versus- host disease in patients aged ≥ 45 years [60, 61].

2.6.2. Prognosis and survival.

Without treatment patients with aplastic anaemia have a high mortality rate of about 70% within one year [62]. Usually, the clinical course is variable with complications due to pancytopenia (infections, bleeding), relapse and clonal evolution. However, with increasing availability of haematopoetic stem cell transplant and effective immunosuppressive therapy, survival rates have increased to as high as 80% [63].

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

2.7.1. Pharmacotherapy

Drug-induced aplastic anemia has long been a menacing outcome of modern pharmacotherapy. The incidence of idiosyncratic, drug-induced aplastic anaemia varies depending on the genetic susceptibility and the associated drug [64]. Only limited studies have explained the epidemiology and actual incidence of this reaction and drugs are the important risk factors believed to be linked with aplastic anaemia. Studies have identified a range of pharmaceutical agents related with development of aplastic anemia. Avoidance of suspected drugs will reduce the incidence of aplastic anaemia and improve the quality of life [65]. There are known associations between aplastic anemia and exposure to carbamazepine with odds of 2.7.

Carbamazepine is the most widely prescribed drug that had a relationship with aplastic anaemia as documented in a different case report [28-30, 66]. Carbamazepine, phenytoin, and phenobarbitone are the first-choice antiepileptic drugs. Regardless of the availability of newer antiepileptic drugs, these drugs are widely used because of their effectiveness and low cost. All 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 with the use of other antiepileptic drugs like sodium valproate, phenytoin, and felbamate, were found in several case reports.

A strong relationship has been documented in a study in Thailand, which showed that the risk of aplastic anaemia increased with 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 has been the most common cause of aplastic anemia and is attributed to 20% to 30% of total cases [68, 70]. In the United States, about 50% of aplastic anaemia cases are known to be caused by these drugs from 1949 to 1952 [71]. Globally, despite its effectiveness, low cost, and few numbers of side effects, the use of chloramphenicol has been limited. However, withdrawal from markets was not followed by a decline in the incidence of aplastic anaemia [71]. Based on this discussion, it was accepted with the view of Surapol Issaragrisil et al. [70-73], that aplastic anaemia risk among chloramphenicol users still needs further investigations.

2.7.2. Mebendazole

Mebendazole, an anthelmintic drug, is of particular interest because it appears to be significantly connected with aplastic anaemia and similar association was confirmed globally [72]. In Pakistan, herbal medicines or home remedies used are common because people believe that they might be effective in the treatment of different diseases. These herbal medicines are formulated with well-known or unknown drugs or chemicals [46, 73] that might be associated with aplastic anaemia [74].

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

This drug is a bacteriostatic antimicrobial medication used for the treatment of a variety of bacterial infections with a trade name Bactrim, and has many reported skins and haematologic side effects [75]. Due to its ~~the~~ easy availability and cost effectiveness, TMP-SMX is one of the medications commonly used for 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 is a reduction in all cell lines [76]. There is a well-known association between this drug and other related drugs such as carbamazepine, thiazides, and mebendazole. The risk of this idiosyncratic reaction differs for different individuals. It is suggested that clinicians be aware of the possibility of drug-related aplastic anaemia and revise their ~~prescribing~~ prescription habits accordingly [77]. There is an urgent need to develop the capacity building of clinicians for continuous surveillance of the safety and efficacy of the pharmaceutical products [78].

2.8. Eltrombopag: a review of its use in patients with severe aplastic anaemia

Eltrombopag (Promacta®) is an orally active thrombopoietin receptor agonist recently approved in the US for the treatment of patients with severe aplastic anaemia who have had an insufficient response to immunosuppressive therapy [68]. Eltrombopag does not compete with thrombopoietin and binds to a different site on the receptor, producing additive effects. It stimulates haematopoietic stem cells and promotes haematopoietic recovery in patients with aplastic bone marrow. Eltrombopag increased platelet counts and can also increase red blood cell and neutrophil counts [67, 69]. In patients with severe aplastic anaemia refractory to prior immunosuppressive therapy, oral eltrombopag at dosages ≤150 mg once daily for 12-16 weeks produced a haematological response in at least one cell lineage in 40 % of patients. Trilineage responses were achieved in nearly one-half of the responders during extended treatment. In robust responders, stable haematological counts were maintained after eltrombopag discontinuation. Eltrombopag was generally well tolerated, with increased liver transaminases as the only dose-limiting toxicity [66, 67]. Clonal cytogenetic abnormalities were observed in 19 % of patients and dysplasia in 5 % of patients.

3.0. HERBAL PRODUCT APPLICATION IN ANAEMIA MANAGEMENT.

Patients in West Africa where sickle cell anemia (SCA) is endemic have for ages been treated with natural products, especially herbs, as, is still the case in rural communities. Studies ~~has~~ have been done on some of these herbs to see if there are any lessons to be learnt or clues to be found for optimizing the treatments based on them [76, 80]. The study revealed that during the last 2-3 decades, much progress has been made in several aspects of anaemia pharmacology, especially the approval of hydroxyurea [78]. As for anaemia herbalism has been revealed that antisickling herbs are common in West Africa and that the most promising are yet to be found. Three new antisickling herbs (*Entandrophragma utile*, *Chenopodium ambrosioides*, and *Petiveria alliacea*) ~~has~~ have been reported [76, 77]. The study raised the hope that the search in the Tropics for more effective herbal recipes for managing aplastic anaemia will be more fruitful with time and effort.

There are several reviews published summarizing the effects of different herbal extracts and their isolated bioactive compounds on human and other mammalian stem cells isolated from different sources. Studies done in 2016 elaborate on osteogenic, anti-adipogenic, neurogenic, endothelial/vascular genesis, angiogenesis and proliferative effects of herbal extracts on human mesenchymal stem cells (hMSCs), that was mostly confirmed by RNA expression studies [80].

UNDER PEER REVIEW

Dried root of Korean herb *Dipsacus asper* 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*, present in Aloe latex, showed anti- adipogenic activity on hMSCs by reducing expression levels of mRNAs (resistin, adiponectin, aP(2), lipoprotein lipase, PPARγ and tumour necrosis factor-α) involved in adipogenic pathways [77]]. Treatment of adipose-derived hMSCs with dried root extract of *Angelica sinensis*, an herb used in traditional Chinese medicine, resulted in significantly higher differentiation of neural-like cells than a commonly used neural inducer, butylated hydroxyanisole [80]. The neuroprotective ability of the same extract was proven by decreased induced neurotoxicity in cultured cortical neurons, increasing the extract's value as a potential candidate ~~in~~ for treating neurodegenerative disorders [77, 80].

4.0. PHARMACOECONOMICS OF THERAPEUTIC INTERVENTION FOR APLASTIC ANEMIA

The best treatment for aplastic anemia is ~~the~~ a bone marrow transplant. A bone marrow transplant is the only cure for aplastic anemia. However, bone marrow transplants are also called stem cell transplants [83]. A transplant is the preferred treatment for severe aplastic anemia. Bone marrow transplants replace damaged stem cells with healthy ones. ~~An~~ 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; which can lead to a response rate of 60 to 70% [75].

Having ATG is rather easy, it is injected into a 1000 ml bag of normal saline, and attached to the cell line or Hickman line, and allowed to run for around 12 hours a day, the course of treatment is normally 4 days. Before having the first full bag, a small dose is given over an hour as some patients may have allergic responses [66]. Therefore, idiopathic aplastic anemia is a diagnosis of exclusion. The clinical severity of aplastic anemia is classified based on the peripheral blood counts and results of bone marrow examinations. The signs and symptoms of aplastic anemia are primarily those associated with pancytopenia. Immunosuppressive therapy (IST) has demonstrated low response, toxicity, and risk of transformation [67]. In a Phase I/II trial, the addition of eltrombopag to first-line IST 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 ± eltrombopag and were followed for 1 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 (low-dose cyclosporine), and non-responders received 6 months of second-line eltrombopag monotherapy [68].

Costs considered included first-line, maintenance, and second-line therapy, administration, routine care, mortality, and adverse events (AEs) [65]. All cost data were reported in 2018 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 + 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.

5.0. APLASTIC ANEMIA IN CHILDREN

Aplastic anemia is a serious condition in which the bone marrow does not make enough new blood cells. With fewer blood cells, a child with aplastic anemia has less oxygen sent to organs, tissues, and cells from too few red blood cells [81]. They have an increased risk of infection (from too few white blood cells), and an increased risk of bleeding problems (from too few platelets). Aplastic anemia in children has many causes [86]. Sometimes the cause is unknown. There are many known causes. Aplastic anemia may develop ~~it~~ at some point during childhood. Or it may be passed down from parent to child.

5.1. Acquired causes of aplastic anaemia in children include:

Infection. These include hepatitis or liver infection, and many different viral illnesses, such as Epstein-Barr virus (EBV), cytomegalovirus (CMV), parvovirus B19, or human immunodeficiency virus (HIV) [88].
Cancer. Some cancers affect the bone marrow.
Autoimmune disease. These include lupus and rheumatoid arthritis.
Medicines. This includes some antibiotics and other medicines.
Toxins. These include heavy metals, pesticides, and benzene.
Radiation therapy and chemotherapy. These are done to treat cancer.

5.2. The symptoms of aplastic anemia in children.

The most common symptoms of aplastic anemia are;

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

5.3. Diagnostic of aplastic anemia in children

Children healthcare provider will likely refer a child to a hematologist, an expert in blood disorders. Along with a complete medical history and physical examination of children, tests for aplastic anemia may include [81]:

Hemoglobin and hematocrit. This blood test measures the amount of hemoglobin, the part of red blood cells that carry oxygen, and red blood cells in the blood.
Complete blood count (CBC). A complete blood count checks the red and white blood cells, blood clotting cells (platelets), and sometimes, young red blood cells (reticulocytes). It includes hemoglobin and hematocrit and more details about the red blood cells.
Peripheral smear. A small sample of blood is examined under a microscope. Blood cells are checked to see if they look normal or not.
Bone marrow aspiration or biopsy. This procedure is done by taking a small amount of bone marrow fluid (aspiration) or solid bone marrow tissue, called a core biopsy. The hip bone is often used. 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.

Treatment will depend on a child's symptoms, age, general health, and how severe the condition is. Treatment for aplastic anemia also depends on the cause. For mild aplastic anemia, treatment may not be needed or treatment may include: Blood transfusions, Platelet transfusions, Antibiotics, Hormones or other medicines (to stimulate the bone marrow to produce cells), Immunosuppressive medicine, Stem cell transplant [65, 82].

During treatment, some possible complications may result. With correct treatment, the risk of complications may reduce ~~with~~ in aplastic anemia. The complications of aplastic anemia may include: ~~Medicine used to treat anemia may cause~~ Treatment-related side effects, Problems with growth and development, Cancers, Heart failure, Uncontrolled bleeding, and Severe infections [73, 84].

Primary parental intervention ~~of~~ in children with aplastic anemia

There is a need to work with a children healthcare provider to develop a treatment plan, and normalize children's life as possible. Efforts should be made to pay attention to 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. A child may also qualify for special rehabilitation programs in some well-developed countries. For kids who feel different or alone, it is important to find a support group for children with anemia. Kids affected must not do activities that increase the chance of infection or bleeding. Such activities include things like: Staying away from people who are sick, eating uncooked foods, contact sports (for example, football, hockey, skiing, or rollerblading) and ~~avoid~~ traveling to high altitudes (children with a low red blood cell count will have increased fatigue and need for oxygen in high altitudes) [88]. How can staying away from people who are sick be classified as activities to avoid? Or do you mean children with aplastic anaemia should

not be allowed to come into contact with sick individuals? Please, clarify by rephrasing the statement.

Conclusion

The treatment of severe aplastic anemia, whether by allogeneic stem cell transplantation or immunosuppression, has improved dramatically over the years, and long-term survival of more than 75% of patients can be anticipated with either therapy. A multidisciplinary approach is recommended to manage relevant results and develop a treatment plan. Consideration should be given to seeking ~~an~~ expert advice on the diagnosis and management of patients where there is uncertainty, or when an inherited bone marrow failure syndrome is being considered and more encouragement for participation and enrollment in clinical trials for drug testing for AA. There is a need for clinicians to be aware of the possibility of drug-related aplastic anemia and revise their ~~prescribing~~ prescription habits accordingly. There is an urgent need to develop the capacity building of clinicians for continuous surveillance of the safety and efficacy of the pharmaceutical products which are used in their clinical practice for aplastic anaemia interventions. The formulation of new legislation for drug safety and national guidelines for pharmacovigilance in AA drug adverse effects and safety pharmacology is highly recommended. A general sensitization advocacy is needed for the risk of auto medication in the population on target antibiotics use that are of potential predisposition risk to aplastic anaemia

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