Aplastic anemia: Insights and updates

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Abstract
Aplastic anemia (AA) is a rare condition characterised by reduction in production of blood cells due to damaged bone marrow function. In AA, there is autoimmune response towards the bone marrow cells that results in its impairment. AA pathophysiology revolves around upregulation of T cells and pro-inflammatory and inflammatory cytokines. AA has multiple causes and pathogenic factors, and all lead to destruction of hematopoietic stem cells. Many molecular proteins and cascades are associated with pathological advancement in AA. Diagnosis of AA is also challenging. AA is generally diagnosed by blood cell count and haemoglobin determination. However, for differential diagnosis various protein markers for peripheral blood can be utilised. Therapeutic considerations for AA mainly include haematopoietic stem cell transplant and immunosuppressants. However, nowadays, may novel therapies as well as new regimens are discovered. Such novel approaches prove promising for therapeutic advancement and possible cure for AA. Extensive studies through collaborative efforts in this area can lead to better therapies for mitigation of AA.

1. Introduction
Aplastic anemia (AA) is a rare condition in which there is unwanted immune response towards haematopoietic cells that result in impaired bone marrow function and less production of peripheral blood cells (1,2). About 32.9% people were found to suffer from AA worldwide up to 2010. Children (up to 5 years), infants and pregnant females were more susceptible to AA. It is generally observed that risk of AA was more in female than male population. It was also observed that AA risk increased with increasing age in geriatric people (3). Additionally, it was seen that AA prevalence was high in Asian countries as compared to western countries like America. Contrary to previous findings, in an epidemiological study it was observed that prevalence of AA was higher in male than in female. Out of 1324 patients, 997 patients of AA were male whereas 327 were female patients. Cases of severe and very severe AA were comparatively higher than non-serious form of the disorder. It was also found that majority of patients belonged to low socioeconomic status (4). T-cells are abnormally activated which result in decreased hematopoietic stem cells and progenitor cells (5). Clinical manifestations of AA include fatigue, dyspnoea, bruising, gingival bleeding, heavy menstruation, fever and headache (1).

1.1 Pathogenesis

In patients of AA, it is observed that expression of T-box transcription factor TBX21 (T-bet) is elevated. Increase in T-bet further leads to imbalance of type-1 and type 2 helper T cells (Th1 and Th2). This consequently leads to upregulation of proinflammatory cytokines that include Interleukin-2 (IL-2), tumour necrosis factor-α (TNF-α), and Interferon-γ (IFN-γ). Furthermore, these processes collectively lead to negative regulation of haematopoiesis and Fas mediated apoptosis of haemopoietic stem cells. Additionally, regulatory T cells (Tregs) are also impaired in AA. These also leads to increase in T helper 17 cells (Th17) and upregulation of retinoic acid receptor related orphan receptor gamma (RORγt) and inflammatory cytokines. Other than this, mutations in Janus-Kinase (JAK)- signal transducer and activation of transcription (STAT) and mitogen activated protein kinase (MAPK) signalling cascades also lead to clonality of CD8+ T cells. Production of cytotoxic T cells is affected due to the presence of defective NK cells (5). It was reported in a study that there was an increase in levels of IL-2, IL-6, IL-8, TNF-α and INF-γ in either peripheral blood or serum of AA patients. Upon administration of mesenchymal stem cells, it was found that levels of TNF-α, IFN-γ and IL-2 were reduced, whereas increased levels of transforming growth factor β (TGF-β) were observed. In addition, it was established that CX3C motif chemokine ligand (CX3CL1) and CX3C motif chemokine receptor 1 (CX3CR1) were implicated in pathogenesis of AA. Interaction of these stimulates release of T cells in bone marrow. Increased levels of CX3CL1 were seen in patients of AA. Interestingly, another interleukin IL-21 was also found to be involved in pathophysiology of AA. IL-21 not only suppresses Tregs, but also increases IL-17. Furthermore, it alters the expression of Forkhead box protein 3 gene (FOXP3) (6) (**Figure 1**).

1.2 Diagnosis

Complete blood count, bone marrow biopsy or aspiration, reticulocyte count and leukocyte differential count are commonly used techniques for AA diagnosis (1). Differential diagnosis in AA is quite challenging. However, there are some diagnostic markers associated with AA that include glycosylphosphatidylinositol anchored protein deficient blood cells and HLA class I allele lacking leukocytes. It is also observed that expression of T-cell immunoglobulin and mucin domain 3 (TIM3) is decreased in AA patients. TIM3 marker suggests the maturation of natural killer cells. However, the decreased expression of TIM3 indicate defect in these cells (5). Most common characteristics for diagnosis of AA include presence of two lineage cytopenia. Other hallmarks include haemoglobin less than 10 g/dL, platelet count less than 50 X 109/L and neutrophil count less than 1.5 X 109/L. Severe AA is characterised by neutrophil count lower than 0.5 X 109/L, platelet count lower than 20 X 109/L, reticulocyte less than 20 X 109/L and reduction in normal cellularity of bone marrow to 25% (7) (**Figure 1**).

1.3 Current therapeutics used for AA

Generally, conventional therapeutic strategies in AA involve use of immunosuppressive therapies, blood transfusion and stem cell transplantation (2,5). Transfusion therapy is preferred for patients having multiple comorbidities (5). Anti-thymocyte immunoglobulin (ATG) along with cyclosporine are amongst the most common treatment strategies for AA(2). Haemopoietic stem cell transplant (HSCT) is the foremost approach as transplant therapy. Subsequently HSCT is followed by administration of immunosuppressants like cyclosporine (2). However, HSCT poses a risk for ABO mismatch. In these cases, treatment options include transfusion of red cell units that are compatible to the donor (8). Some other complications can also occur. Minor ABO mismatch also requires transfusion of donor compatible red cell units. Sometimes it is observed that residual plasma cells of recipient cause immune tolerance. In this case, transfusion is preferred. Other transplant options include bone marrow transplant from sibling matched donor (1).

Additionally, administration of rituximab (anti-B cell), donor lymphocyte infusion, erythropoietin, TPO mimetics, Syk inhibitor, intravenous immunoglobulin, daratumumab (anti-plasma cell), bortezomib (anti plasma cell) might be considered. Another complication is development of new autoantibodies along with immune tolerance and mixed chimerism. For this, treatment options include administration of intravenous immunoglobulin, corticosteroids, rituximab, erythropoietin, Sky inhibitor, anti-plasma cell therapy, immunosuppressants, anti-complement or splenectomy (8).

Other than these, thrombopoietin receptor agonists can also be used. These include compounds like eltrombopag, romiplostim, avatrombopag and hetrombopag. These conventionally increase platelet count, however in AA, these drugs stimulate residual hematopoietic stem cells and enhances haematopoiesis (5). Amongst these agents, eltrombopag is widely used in AA.

Androgens are another choice of drug for treatment of AA. These are able to upregulate erythropoietin secretion and haematopoiesis. Additionally, it is observed that there are mutations in telomerase and its RNA component (5). In these cases, androgens also increase activity of telomerase. Androgens and androgen related agents are used which involve testosterone undecanoate, danazol and stanozolol (5,9).

Rapamycin is another agent implicated for treatment of AA. It inhibits mammalian target of rapamycin (mTOR) which has a rather significant role in mitochondrial metabolism of T cells. It also inhibits IL-2 release stimulated by activated T-cells. Alemtuzumab is also used for treatment of AA. It induces depletion of lymphocytes by targeting CD52 and ultimately leads to regulatory phenotype T cells recovery (5). Granulocyte colony stimulating factor (G-CSF) is also used as therapy. It is responsible for expansion of haematopoietic stem cells, inhibits apoptosis, promote differentiation of neutrophils. Some other immunosuppressants like cyclophosphamide, levamisole and mycophenolate mofetil are also used for AA therapy (5) **(Figure 1).**

2. Advancements in AA therapies

Nowadays, unconventional and neoteric approaches with respect to AA treatment are being developed.Similar to HSCT, umbilical cord blood transplantation is one of the unconventional approaches. Initially, cyclosporine was commonly used post-surgery. However, new regiments including agents like melphalan and fludarabine are also used these days (2). There is impairment of telomerase RNA component (TERC) in case of AA. TERC is involved in extension and maintenance of telomeres. TERC haploinsufficiency affects length of the telomer and results in conditions like AA. A new therapeutic strategy, cell reprogramming allows to reverse differentiation of cells and transforms cell into pluripotent stem cells with more ability. Furthermore, programming can affect length of telomer and can extend it. TERC cell reprogramming is thus a promising strategy for therapy of AA. Cells like mesenchymal stem cells and induced pluripotent stem cells (iPSC) can be used for this purpose (10).

In a phase II clinical trial for AA, it was found that avatrombopag along with immunosuppressive therapy in refractory AA patients was effective. The trial was conducted in two cohort groups. The first group consisted of severe AA patients who have not yet received any therapy. Another group consisted of severe AA patients who had refractory or relapse condition after therapy. First group received anti-thymocyte globulin and cyclosporine along with avatrombopag for 180 days. The other group received avatrombopag with or without any additional therapy (11). Another trial involved determination of efficacy of romiplostim in refractory AA patients. Romiplostim is a fusion protein that stimulates thrombopoietin receptor and consequently activates transcriptional pathways that ultimately stimulate hematopoietic cells, megakaryocytes and progenitor cells. Initially the study was conducted for 53 weeks, however

efficacy was not adequate, hence the study was extended up to 3.5 years and closely observed patients who took romiplostim in their regimen. This long-term therapy proved to be effective and relatively safe for therapy of AA (12). Another study evaluated the prophylactic effect of ruxolitinib prior to allogenic stem cell transplantation in 35 patients. Following the transplantation there is a prominent risk of graft versus host disease. To eliminate this possibility, ruxolitinib was administered to reduce the risk of this occurrence. Administration of ruxolitinib exhibited less risk of graft versus host disease, lower infections, restoration of Tregs and CD4+ cells. This shows that ruxolitinib is effective in such conditions (13) (Figure 1).

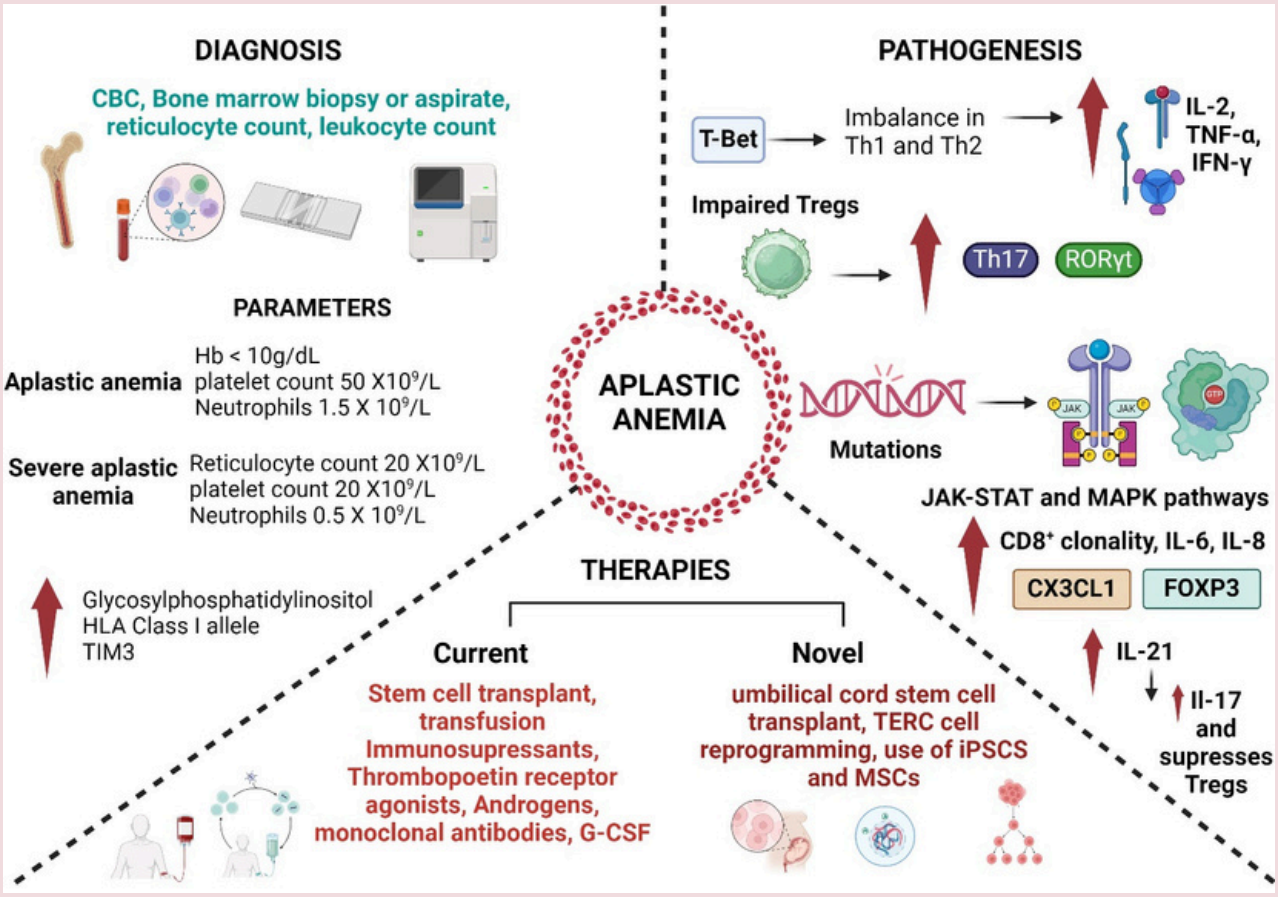

Figure 1. Diagnosis, pathogenesis and therapies for aplastic anemia

3. Challenges and opportunities in AA research

AA is a rare disease and hence, research in this area is scarce. Research should be extensively promoted in this area, as there are many challenges for therapeutic intervention for AA. Stem cell transplantation is the foremost treatment strategy in AA. However, it poses a challenge to find a donor that matches the patient in a time bound manner. To overcome this haploidentical stem cell transplantation technique can be used. This technique has been reported to lower the prevalence of graft failure and occurrence of graft versus host disease. Such techniques can be explored more, and further extensive research should be carried out in these areas to overcome these types of challenges in therapy of AA (14). Another major challenge for mitigation of the disease is the lack of specific treatment for AA patients. This might lead to less chances of survival of the patients. More resources are required to design a specific regimen for AA therapy.

Availability of such resources can prove to be helpful for treatment of AA. Also, it was observed that mortality for severe AA cases also involves factors like presence of other comorbidities like infection. Haemorrhage is also one of the leading causes of death in severe AA. These issues can be minimised through selection of suitable therapeutic alternatives with respect to the condition of patient (15). Government organisations can provide aid for conducting these studies that will probably help in providing remission to AA patients. Aplastic anemia and myelodysplastic disease syndromes (MDS) International foundation is a USA based foundation that promotes research as well as provides primary care to AA patients (16). In India, DKMS-BMST foundation is a non-profit organisation that promotes research and primary care for disorders like AA, blood cancer and thalassemia (17). Such non-profit organisations might help promote and advance research in these rare areas and hence support to such entities should be encouraged. Industry-academia-government collaboration should be highly encouraged to promote research and development of treatment modalities and algorithms for the treatment of AA.

4. Conclusion

AA is a rare blood related disorder in which there is less production of blood cells. It usually occurs through dysfunction in bone marrow due to untoward auto immune response. Diagnosis and therapy in AA pose quite a challenge. However, differential diagnosis of AA is possible through various markers in peripheral blood. It is observed that therapies for AA majorly include stem cell transplant and administration of immunosuppressants. Moreover, nowadays novel techniques like TERC cell reprogramming and other transplants are being developed for clinical use. More emphasis on these novel procedures can lead to successful mitigation of AA.

5. **Acknowledgment**

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