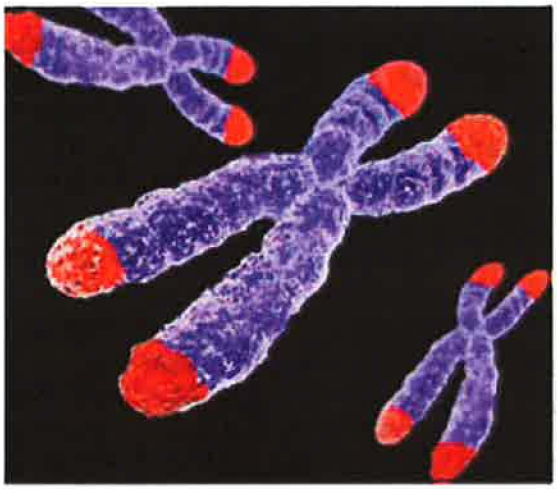
Anemia, Aplasia, and Ageing



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Case Presentation

A 23 year-old Caucasian female with no past medical history presented in July of 2009 with menorrhagia. On physical exam, she had diffuse petechiae, but no other signs of bleeding, no lymphadenopathy, and no hepatosplenomegaly. Her complete blood count showed a white blood cell count of 2.32 X 10^9/L, hemoglobin of 7.0 g/dL (mean corpuscular volume 85.8 femtoliters, red blood cell distribution width 14.6%), and platelets of 11 X 10^9/L. Her differential showed 17% neutrophils (absolute neutrophil count 0.39 X 10^9/L), 76% lymphocytes, 6% monocytes, and 1% eosinophils with no blasts. Her reticulocyte count was 23 X 10^9/L. A chemistry panel was completely normal with a total bilirubin of 0.3 mg/dL and a lactate dehydrogenase of 184 Units/L. Her vitamin B12 level was 305 pg/mL and folate was 13.2 ng/mL. The following serologies were all negative: human immunodeficiency virus-1, human immunodeficiency virus-2, Epstein-Barr virus IgM, monospot, Hepatitis B surface antigen, and Hepatitis C. A serum anti-nuclear antibody and rheumatoid factor were negative. Her urinalysis did not show any protein or blood and a urine pregnancy test was negative. A peripheral blood smear showed a normocytic, normochromic anemia, severe thrombocytopenia, leucopenia, and normal morphology of the polymorphonuclear neutrophils. She was admitted and a bone marrow biopsy was performed that showed a severely hypocellular marrow (< 10%) without abnormal infiltration or increased reticulin. Her cytogenetics were normal (46,XX). Flow cytometry of the bone marrow aspirate showed a large subset of monocytes lacking expression of CD14. Flow cytometry of the peripheral blood cells showed absent expression of multiple glycosylphosphatidyl inositol (GPI)-linked antigens (CD14, CD16, CD24, CD55, and CD59) on granulocytes, monocytes, and erythrocytes. Fluorescent-labeled inactive toxin aerolysin (FLAER) testing showed that she had 99.4% type 1 red blood cells with normal CD59 expression, 0.2% type II red blood cells with partial CD59 deficiency, and 0.4% type III red blood cells with complete CD59 deficiency giving her a diagnosis of severe aplastic anemia with subclinical paroxysmal nocturnal hemoglobinuria (PNH-sc). She had two siblings who were found to be human leukocyte antigen (HLA)-identical so four months after presentation, she underwent a matched related bone marrow transplant from her sister after receiving cyclophophosphamide 50 mg/kg intravenously on days -5, -4, -3, and -2 and antithymocyte globulin (ATGAM) 30 mg/kg on days -5, -4, and -3. This was complicated by mild graft versus host disease (GVHD) of the skin which resolved with steroids and cytomegalovirus reactivation which responded to foscarnet and valganciclovir. Peripheral blood chimerism studies done 2 months post-transplant showed 100% donor derived hematopoiesis.

She did well until 5 months after her bone marrow transplant, when her platelets suddenly began to fall to < 5 X 10^9/L. A bone marrow biopsy showed 40% cellularity and adequate to slightly increased megakaryocytes. A polymorphism analysis showed that she was 100% donor and assays for cytomegalovirus and human herpes virus-6 were negative. She received intravenous immunoglobulin (IVIG) and her platelet count increased from 32 to 52 to 68 X 10^9/L so a diagnosis of immune thrombocytopenic purpura was made. She was treated with steroids and rituximab, but her steroid dose could not be tapered down so she underwent a laparoscopic splenectomy with immediate improvement of her platelet count to 224 X 10^9/L the next day. She is now two years out

from her original diagnosis and nine months post-splenectomy and is currently in remission from her severe aplastic anemia, subclinical paroxysmal nocturnal hemoglobinuria, and immune thrombocytopenic purpura, and doing well.

Introduction

Aplastic anemia is characterized by peripheral pancytopenia and a hypocellular bone marrow (< 10%) in the absence of infiltration or increased reticulin (**Figure 1**). This is a misnomer because patients with aplastic anemia do not only have anemia, but pancytopenia. However, the term was introduced by Chauffard in 1904 and has remained in popular use. These patients are usually divided into those with an inherited bone marrow failure syndrome and those with acquired aplastic anemia. The latter will be the topic of this review.

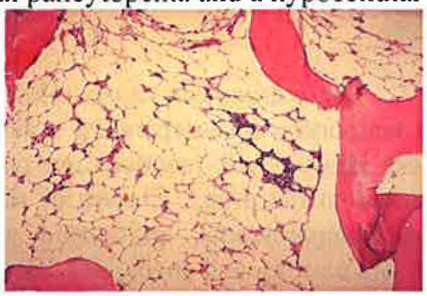

Figure 1: Bone marrow biopsy showing aplasia without infiltration or increased reticulin.

Epidemiology

Aplastic anemia is a rare disorder. The International Aplastic Anemia and Agranulocytosis Study (IAAAS), the largest epidemiologic study of bone marrow failure, reported an incidence of 2 cases per 1 million people.[1] However, this study was performed in Europe and Israel and several smaller studies in the Eastern Hemisphere have shown the incidence to be two to five times higher.[2] The disorder can occur at any age, but there are two peaks in incidence: between ages 10-25 and in patients over 60.[3]

Pathophysiology

Acquired aplastic anemia can be due to a variety of causes. Associations have been made with certain environmental toxins, medical drugs, and viruses. These, however, appear to cause only a minority of cases. Most patients with idiopathic acquired aplastic anemia are felt to have an autoimmune disease with destruction of bone marrow stem cells.

Environmental Toxins

Many toxins have been associated with aplastic anemia. To definitively prove causal association between one particular agent and the disease is difficult. In the past, a precise definition of aplastic anemia did not exist and it was difficult to distinguish this disorder from other causes of marrow failure. The most well known chemical associated with aplastic anemia is benzene. Beginning in the 1900's, reports of benzene-induced aplastic anemia eventually led to a successful campaign to improve safety in the

workplace by replacing it with toluene or naphtha. Nowadays, with modern standards of hygiene in industrial workplaces in the U.S., the risk of most chemicals causing aplastic anemia is usually very low, but there are still reports that exposure to levels of benzene below the U.S. occupational standard of 1 part per million (ppm) can cause hematotoxicity.[4] In underdeveloped countries, where protective measures are not as strictly enforced, an increase in the incidence of aplastic anemia can be seen amongst those of lower socioeconomic status.[5]

Drugs

Some drugs can cause aplastic anemia as an idiosyncratic reaction as opposed to the temporary dose-dependent aplasia seen with chemotherapeutic drugs or radiation. These idiosyncratic reactions are rare, occurring in 1 out of 100,000 – 200,000 people exposed to the drug. Classes of drugs that have been associated with aplasia include immunosuppressants, antithyroid medications, nonsteroidal anti-inflammatory drugs, anticonvulsants, and medicines used to treat tuberculosis.[6] The most commonly cited drug, chloramphenical, is rarely used nowadays. A study in Thailand showed that while drugs were the most identifiable cause of aplastic anemia, they were only implicated in 5% of cases.[7] In most patients, cessation of the drug does not result in recovery of hematopoiesis. Sometimes it can be difficult to distinguish drug-induced aplastic anemia from idiopathic aplastic anemia.

Viruses

As is the case with medical drugs, many viruses have been implicated to cause aplastic anemia, but direct causation is difficult to prove. Some viruses can cause a transient pancytopenia (e.g. Epstein-Barr virus, human immunodeficiency virus, etc) that may later resolve. Hepatitis-associated aplastic anemia is a variant of aplastic anemia whereby patients first present with acute hepatitis, then develop aplastic anemia several months later.[8] It occurs more commonly in Asia[9,10] and usually affects adolescent males.[11] These patients have negative serologic tests for hepatitis A, B, C, D, E, G, and Torque Teno virus (TTV). If untreated, the condition is almost always fatal, but many patients will respond to immunosuppression or allogeneic bone marrow transplantation.[12] Parvovirus B19 usually causes a pure red-cell aplasia because the virus attaches to the erythrocyte P antigen, also known as globoside, but rare cases of aplastic anemia have been reported.[13]

Autoimmunity

In the majority of patients with aplastic anemia, an identifiable cause cannot be determined. Most of these patients respond to immunosuppressive therapies.[14] It has been shown that in patients with aplastic anemia, cytotoxic T lymphocytes attack the marrow stem cells by expressing Th1 cytokines, especially gamma interferon (**Figure 2**).[15] They also triggers Fas-mediated apoptosis of stem cells (**Figure 3**).[16-18] The autoantigen that trigger the T cells to become activated is not entirely clear. By screening antibodies in the sera of patients with the disorder against a peptide library of fetal liver

cells, two possible antigens have been identified: kinectin and anti-postmeiotic segregation increased 1 (PMS1).[19] Antibodies directed against these antigens are only detectable in patients with aplastic anemia and are no longer detectable when the patients are in remission. However, a study by the Japan Childhood Aplastic Anemia Group did not show that anti-PMS-1 antibodies disappeared as patients responded to immunosuppressive therapy.[20]

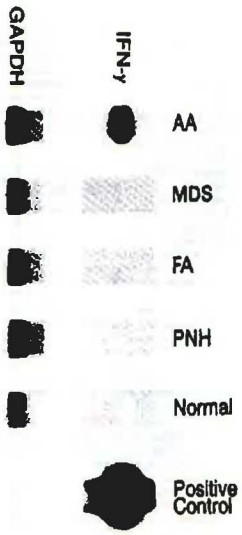

Figure 2: Gamma Interferon expressed by cytotoxic T lymphocytes in patients with aplastic anemia, but not other disorders that cause pancytopenia [14]

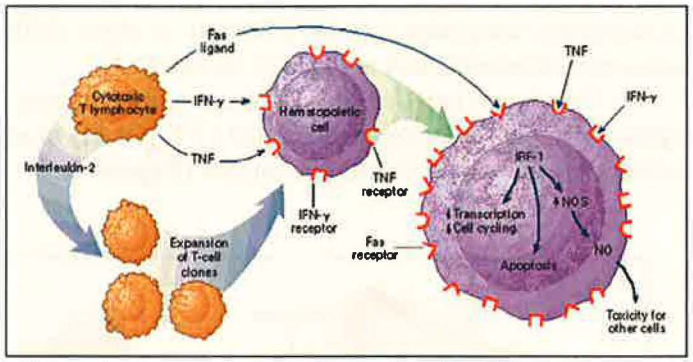

Figure 3: Fas-mediated apoptosis trigged by cytotoxic T lymphocytes [18]

Like other autoimmune diseases, there is association with certain histocompatibility locus specificities. Patients with aplastic anemia have the histocompatibility locus human leukocyte antigen (HLA) DR2 twice a commonly as the normal population.[21]

Telomerase

In 1975, a rare inherited form of bone marrow failure called dyskeratosis congenita (also known as Zinsser-Cole-Engman syndrome) was described.[22] Patients with this disorder have abnormalities in two tissue types beginning in infancy. One is the epithelium, manifested by dystrophic nails, reticulate skin hyperpigmentation, and oral leukoplakia, and the other is the bone marrow. By the age of 10-20, the bone marrow begins to fail and over 80% of patients with dyskeratosis congenita die at a median age of 16 of aplastic anemia. Based on these clinical findings, these patients seem to have dysfunction of stem cells causing problems in cells with a high turnover rate such as skin, oral mucosa, and bone marrow. They also demonstrate early signs of aging including damaged teeth, premature graying of the hair, abnormalities in a wide variety of organ systems, and an increased risk of malignancy.[22,23] In the late 1990's it was discovered that patients with dyskeratosis congenita have extremely short telomeres (< 1st

percentile).[24,25] This led to the discovery of mutations in a gene called DKC1 that encodes a protein called dyskerin which is part of the telomerase complex. More mutations were later discovered and it is now known that almost all patients with dyskeratosis congenita have accelerated telomere shortening due to mutations in genes that encode various components of telomerase or telomere-binding proteins. Dyskeratosis congenita can be inherited in three different patterns: X-linked recessive, autosomal dominant, and autosomal recessive.[23]

Telomeres cap the ends of linear chromosomes and are composed of 500 to 2000 repeats of a particular hexanucleotide (TTAGGG) along with several protective proteins collectively called the shelterin complex (**Figure 4**).[26]

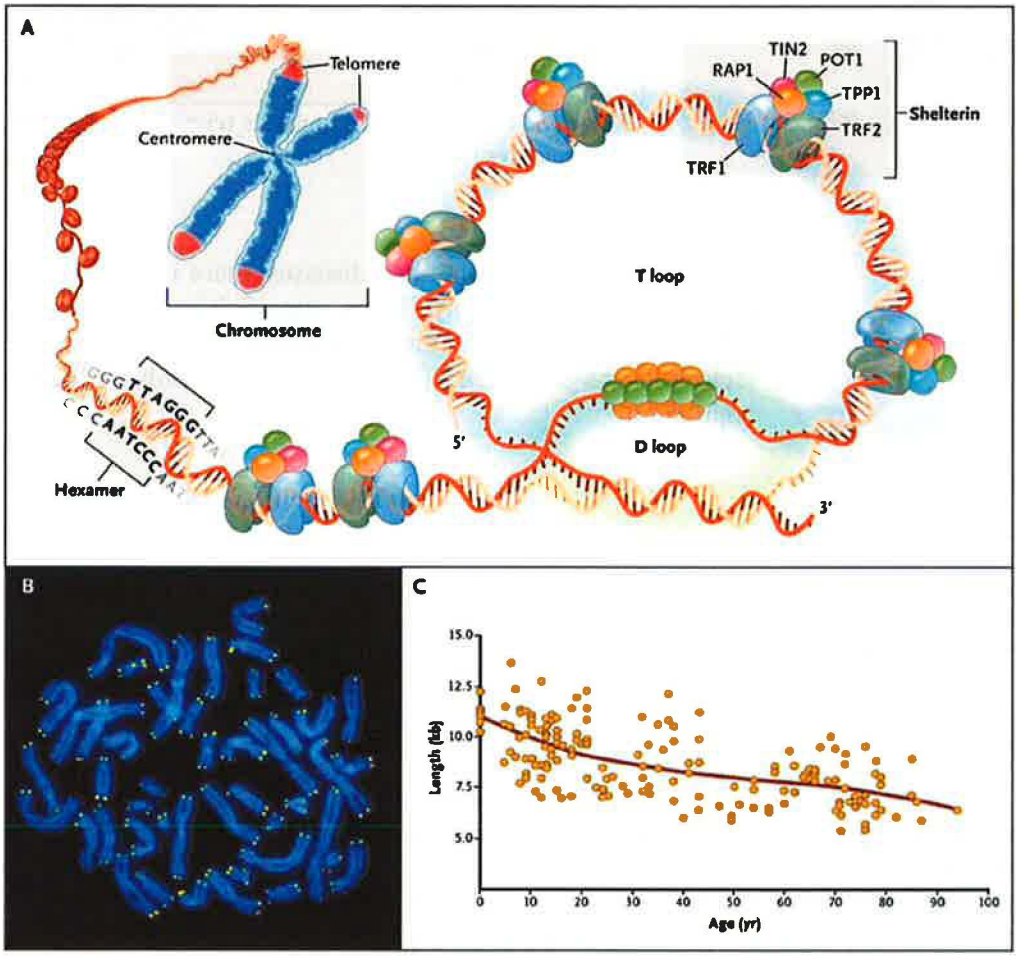

Figure 4: Structure of Telomeres [26]

The telomerase enzyme has two components: a catalytic subunit called TERT (telomere reverse transcriptase) and an RNA template encoded by TERC (**Figure 5**).[27] TERC binds to several other proteins, one of which is dyskerin, a protein important in the stabilization of the telomerase RNA-protein complex. Patients with X-linked recessive cases of

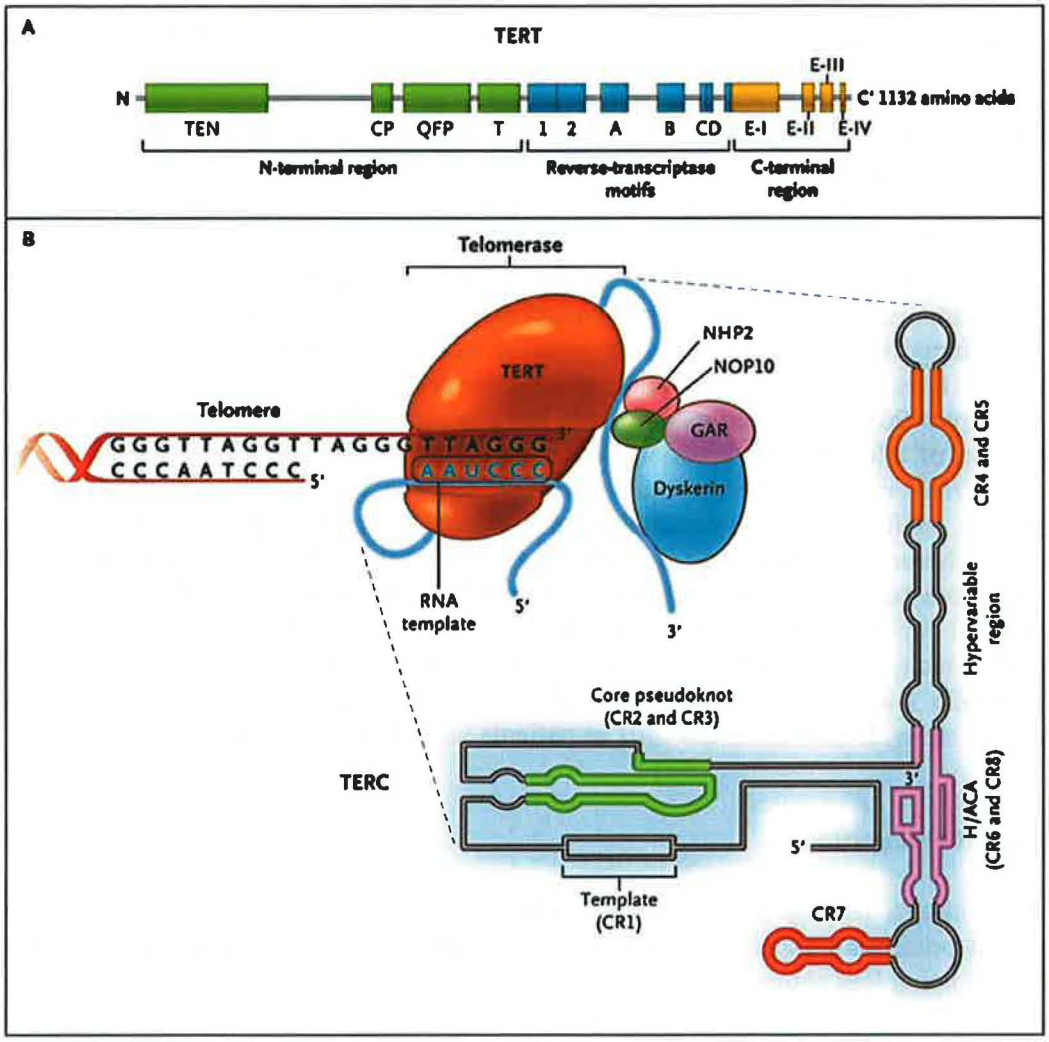

Figure 5: Components of Telomerase [26]

dyskeratosis congenita have mutations in the DKC1 gene and the autosomal dominant forms are due to mutations in hTER, hTERT, and TINF2.[28,29]

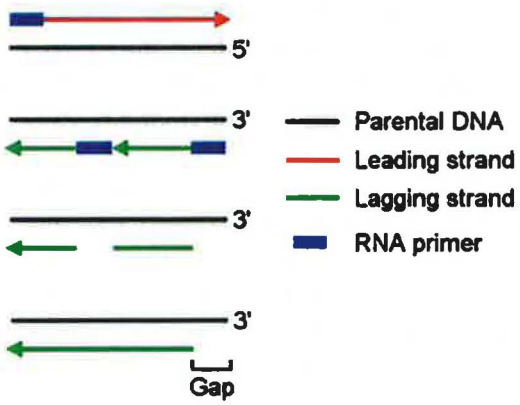

Figure 6: End replication problem

Each time cells divide, 50-100 base pairs will be lost from the 3' end[30] because, as James Watson discovered, "there was no simple way for 3-5' growth to reach the 3' end of its template". This became known as the "end-replication problem" (**Figure 6**).[31]

Telomerase is normally expressed in embryonic and adult stem cells, and highly proliferative cells such as mature lymphocytes. Cells that do not express telomerase (e.g., most mature adult cells) will have progressive shortening of their

telomeres with each division, a process that has been termed telomere attrition. Once this attrition reaches a critical point, even in one single chromosome, the cell dies.[32] Somatic cells in culture can only replicate a finite number of times. The exact number is called the Hayflick limit.[33] However, if telomerase is introduced into these cells, they do not demonstrate senescence.[34]

The telomeres of patients with idiopathic aplastic anemia are much shorter than in age-matched controls.[35] Telomere lengths also correlate with relapse after immunosuppressive therapy, clonal evolution, and mortality in patients with severe aplastic anemia treated with immunosuppression.[36] Those who do not respond to immunosuppressive therapies may respond to androgen therapy[37] because steroid sex hormones have been show to stimulate telomerase activity.[38]

Over 90% of human cancer cells have high levels of telomerase activity[39] and inhibition of telomerase expression limits tumor cell proliferation.[40] Loss of telomere function may result in chromosome rearrangements in cancer cells and lead to tumor growth.[41] GRN163L or imetelstat is a lipidated 13-mer oligonucleotide N3' P5'-thiophosphoramidate that binds to TERC and is a potent inhibitor of telomerase. Phase I studies have been completed and the compound is being evaluated in phase II studies in both solid and hematopoietic malignancies. [42,43] Work is also being done on telomerase peptide vaccines. GV1001 is a synthetic peptide that correspondes to hTERT residues 611 to 626 and is being studied in a phase III randomized trial of gemcitabine and capecitabine with or without GV1001 in patients with metastatic pancreatic cancer. [43,44]

Clinical Presentation

Patients usually present with problems related to their cytopenias. The most common presenting signs and symptoms are fatigue and hemorrhage, with neutropenic infection being less common.

Diagnosis

Diagnosis is usually made by bone marrow biopsy after other causes of pancytopenia have been evaluated. Acquired aplastic anemia overlaps with other bone marrow failure syndromes including myelodysplasia and paroxysmal nocturnal hemoglobinuria (PNH) so one should always evaluate for a concomitant diagnosis of PNH.[45] Interestingly, it has been shown that having detectable PNH cells conferred a favorable prognosis among patients with aplastic anemia and was predictive of a response to immunosuppressive therapy.[46]

Once the diagnosis is made, patients with aplastic anemia are divided into those with severe disease, very severe disease, and nonsevere disease. In all three cases, bone marrow cellularity must be < 25%. If patients meet two of the following three criteria, they have severe disease: absolute neutrophil count < 500 at diagnosis, platelets < 20K at diagnosis, and reticulocyte count < 20K at diagnosis. If they meet these criteria, but the absolute neutrophil count is < 200, they are considered to have very severe disease.

Patients without severe or very severe disease are considered to have nonsevere aplastic anemia.

Treatment

Bone marrow transplantation (BMT)

In 1972, the first successful allogeneic bone marrow transplant was performed on a patient with aplastic anemia.[47] Since that time, long-term results have steadily improved to a 75-80% cure rate due to advancements in the selection of human leukocyte antigen (HLA)-matched donors, supportive care, and conditioning regimens (**Figure 7**).[48]

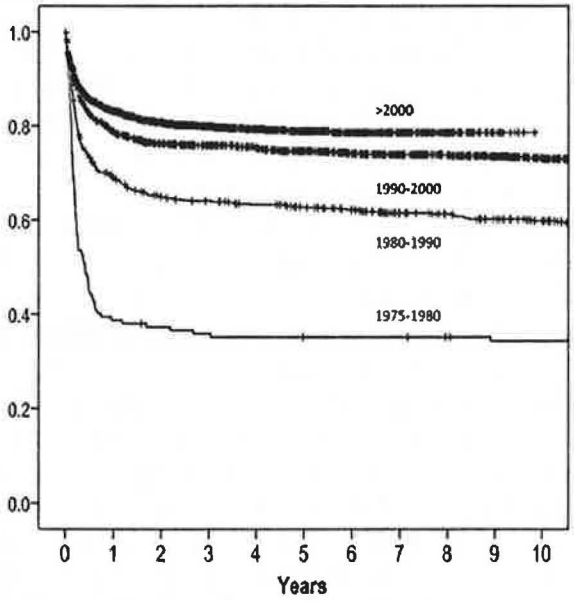

Figure 7: Overall survival after bone marrow transplantation for aplastic anemia [48]

Children under 16 years of age have an even better survival of 91%.[49] Regarding the stem cell source [bone marrow cells versus granulocyte-colony stimulating factor (G-CSF) mobilized peripheral blood stem cells (PBSCs)], a retrospective combined Center for International Blood and Marrow Transplant Research (CIB-MTR) and European Group for Blood and Marrow Transplantation (EBMT) study showed that PBSCs result in earlier engraftment, no difference in graft rejection, but decreased survival due to increased chronic graft versus host disease.[50] In patients < 20 years of age, survival decreased from 85% to 73% with the use of PBSCs and in patients > 20 years of age, survival decreased from 64% to 52%. Regarding donor selection, a retrospective study by the EBMT showed that survival was improved if the donor and recipient were of the same gender. Male recipients of female donors had more acute graft-versus-host disease (GVHD) and female recipients from male donors had increased graft rejection.[51] The most pressing problems with allogeneic transplantation at this time are the graft rejection rates of 4-14%, a 30-40% chance of developing chronic GVHD, and poor outcomes in older adults.[48]

The best conditioning regimen is controversial. The Working Party on severe aplastic anemia (WPSAA) recommends that younger patients receive cyclophosphamide 50 mg/kg/day for four days plus anti-thymocyte globulin as a conditioning regimen. This is nonmyeloablative, but very immunosuppressive to prevent graft rejection and GVHD. Older patients (those over 30 or 40 years of age) do not do as well with this regimen so some recommend reducing the dose of cyclophosphamide and adding fludarabine.[52] In Japan, the recommended conditioning regimen is fludarabine 100 mg/m2 plus

cyclophosphamide 3000 mg/m2 plus rabbit anti-thymocyte globulin (5 or 10 mg/kg) plus 3 Gy total body irradiation (TBI).[53] Currently, a CTNN study is trying to determine the optimal dose of cyclophosphamide (1, 50, 100, or 150 mg/kg) in combination with fludarabine, anti-thymocyte globulin, and 2 Gy total body irradiation. The 0 mg cohort was discontinued due to rejection and the 150-mg cohort was discontinued due to excessive toxicity. An EBMT analysis of patients with severe aplastic anemia undergoing an HLA-identical sibling BMT showed that total lymphoid irradiation resulted in inferior outcomes.[49]

In the Western world, it is recommended that patients receive cyclosporine and methotrexate for GVHD prophylaxis.[54] This results in a survival advantage compared to cyclosporine alone (84% versus 75%). In Japan, the recommended combination is tacrolimus and methotrexate.[55]

The Johns Hopkins group evaluated high-dose cyclophosphamide without stem cell rescue. 67 patients with severe aplastic anemia were treated with high-dose cyclophosphamide (50 mg/kg/day for four days). At 10 years, the overall survival was 88% and the event-free survival was 58% in 44 previously untreated patients.[56] However, a phase III prospective randomized trial designed to compare the response rate of immunosuppression with either high-dose cyclophosphamide (50 mg/kg/day for four days) plus cyclosporine or anti-thymocyte globulin (40 mg/kg/day for four days) plus cyclosporine had to be terminated prematurely because of three deaths in the cyclophosphamide arm. An intent-to-treat analysis of 31 patients showed no differences in overall response rates, but increased morbidity (e.g. invasive fungal infections) and mortality in the cyclophosphamide group.[57]

Many patients have mixed chimerism posttransplant. The EBMT divides these patients into 5 groups: (1) complete donor chimeras, (2) transient mixed chimeras, (3) stable mixed chimeras, (4) progressive mixed chimers, and (5) recipient cells with early rejection.[58] Patients in the first group have more GVHD whereas those with progressive mixed chimeras have high risk for graft failure. About 30% of patients will have permanent mixed chimeras. These patients have normal cell counts and low rates of GVHD. Graft failure or rejection occurs in the range of 1-25% depending on the conditioning regimen and the transfusion history prior to transplant. Rejection has been classified into several forms: (1) Primary graft failure; (2) Classic early rejection after initial engraftment; (3) Late progressive graft failure; and (4) Acute rejection after discontinuation of cyclosporine. Current studies are investigating new conditioning regimens for second transplants in patients with graft failure.[59]

Results with unrelated donor transplants are improving due to advancements in HLA matching and less toxic conditioning regimens.[60] Survival has increased from 38% in the 1990's to 65% in the early 2000's.[49,60] The results in children are even better with survival rates of 75% versus 63% in the past in children under 16 years of age. Because of these advancements, some recommend that for patients without an HLA-identical sibling donor, an unrelated search should be initiated at diagnosis and an unrelated transplant should be considered after one course of immunusuppression if a suitable donor is found. For those without a suitable unrelated donor, a cord blood transplant can also be considered. A Japanese study of 31 patients who underwent a cord blood transplant showed an overall survival of 42%. However, those receiving a conditioning regimen consisting of fludarabine, cyclophosphamide, and 2Gy of total body irradiation

had an 80% overall survival.[43] Because of the high rates of rejection and low cell numbers in cord blood, this is not to be considered first line therapy, but for those without other options, improvements made with double units[43,61], and new conditioning regimens make this a viable alternative.

Immunosuppressive therapy (IST)

In 1899, Metchnikoff et al., showed that serum from guinea pigs immunized with lymphocytes from mice would cause profound lymphopenia when injected back into the mice. More than half a century later, techniques were developed to immunize animals, usually horses and rabbits and, less commonly, goats, with human T lymphocytes. Antithymocyte globulin is the purified immunoglobulin G fraction of sera obtained from these immunized animals. Response to antithymocyte globulin occurs in 50% of patients by 3 months and 75% of patients by 6 months.[62] For patients with no response, or those who respond then later relapse, a second course of antithymocyte globulin given no sooner than 3-6 months later may result in a response. In patients who failed to respond to or relapsed after horse antithymocyte globulin, treatment with rabbit antithymocyte globulin resulted in a response in 30% of initial nonresponders and 65% of patients who relapsed.[63] The different antithymocyte globulin preparations available for clinical use have never been compared in a randomized setting so care must be taken when interpreting data because it is unknown if results obtained with one preparation would be the same with the other.[64]

The German Aplastic Anemia Study Group performed a randomized trial of antilymphocyte globulin and methylprednisolone versus antilymphocyte globulin and methylprednisolone plus cyclosporine in 84 patients not eligible for bone marrow transplant.[65] An 11-year follow-up of this trial showed a higher response rate in all patients (70% versus 41% in favor of the cyclosporine group, P = 0.15), in patients with severe aplastic anemia (65% versus 31% in favor of the cyclosporine group, P = 0.11), faster time to response (median 60 versus 82 days, in favor of the cyclosporine group, P = 0.019), and a decreased need for repeated courses of immunosuppression.[66] If cyclosporine is withdrawn sooner than 6 months, 30-35% of patients will relapse. A more prolonged course of a year with slow tapering reduces the risk of relapse to 13-16%. One third of patients may become dependent on a small dose of cyclosporine for a longer period.[67]

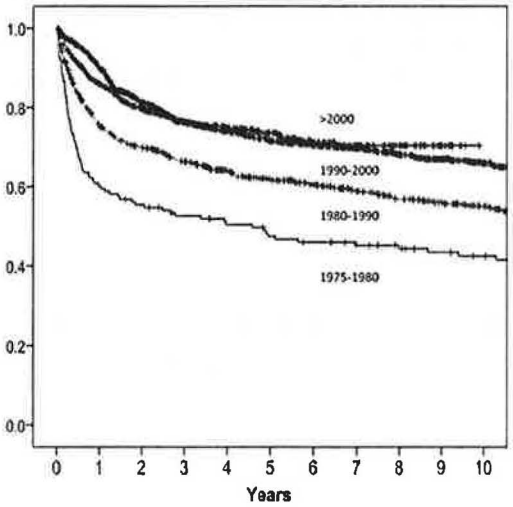

Figure 8: Overall survival after immunosuppression for aplastic anemia [48]

Currently, immunosuppressive therapy with ATG and cyclosporin result in response rates of 60-80% and 5-year survival rates of 75% (**Figure 8**).[68-71]

Lack of response to immunosuppression is likely due to either insufficient immunosuppression,

irreversible stem cell deficiency, or a nonimmune mediated mechanism of the underlying aplasia.

In one study of 316 patients with severe aplastic anemia who received immunosuppressive therapy, higher baseline absolute reticulocyte count and absolute lymphocyte count predicted a response at 6 months.[72] This suggests that the number of stem cells at diagnosis determines response to immunosuppression. Patients in the lowest quartile of baseline telomere length will respond to immunosuppression, but will relapse at twice the rate of patients with normal baseline telomere lengths.[67]

Attempts have been made to add further immunosuppressant agents to the combination of antithymocyte globulin and cyclosporine. A retrospective study showed no benefit with the addition of mycophenolate mofetil[73] and a prospective study showed no difference in response with the addition of sirolimus.[74]

Therefore, in summary, immunosuppression with antithymocyte globulin and cyclosporine is indicated as first-line treatment in patients with nonsevere aplastic anemia who are transfusion dependent, patients with severe aplastic anemia who are over 40 years of age, and patients with severe aplastic anemia less than 40 years of age who lack an HLA-matched sibling donor. Due to the lower cost, increased tolerance, and greater ease of giving immunosuppressive therapy, some have advocated treating all patients with immunosuppressive therapy first, then reserving BMTs for those who fail immunosuppression. Unfortunately, studies have shown that this tactic increases the chance of graft rejection and worsens outcomes.[75] In one study, the hazard ratio for mortality was 1.7 when compared with patients who received a BMT as first-line therapy.[76]

Granulocyte-colony stimulating factor (G-CSF)

Four trials have evaluated the role of G-CSF in patients receiving immunosuppessive therapy.[77-80] G-CSF improves the time to recovery of neutrophils, but has not been shown to have an effect on the response rate, incidence of infection, or overall survival. Of concern is that a European survey of 840 patients who had received first-line immunotherapy with or without GCSF showed a 10.9% incidence of developing MDS/AML in patients who received GCSF as opposed to a 5.8% incidence in those who did not.[81] A current European study has randomized patients to immunosuppresion with or without GCSF.

Androgens

Androgens have been used for many years to treat aplastic anemia without a clear mechanism of action. Aromatase is an enzyme that catalyzes the conversion of androgens to estradiol. It has recently been shown that estradiol increases telomerase activity[82] so for patients with telomere attrition as a cause for their marrow failure, this may be the mechanism of action.

Transfusion Support

Transfusion-associated graft-versus-host disease (TA-GVHD) occurs when T lymphocytes in the donor blood product mount an immune response on the immunocompromised host. Although rare, the condition has a high mortality rate. Therefore, it is recommended that patients with aplastic anemia receive irradiated red cell and platelet transfusions.[83]

Prognosis

The Center for International Blood and Marrow Transplant research recently reported the outcomes of 10,632 patients who had undergone an allogeneic stem-cell transplant for a variety of disorders and survived at least two years. Of the 2,171 patients with severe aplastic anemia, the 10-year survival rate was 92% (**Figure 9**).[84]

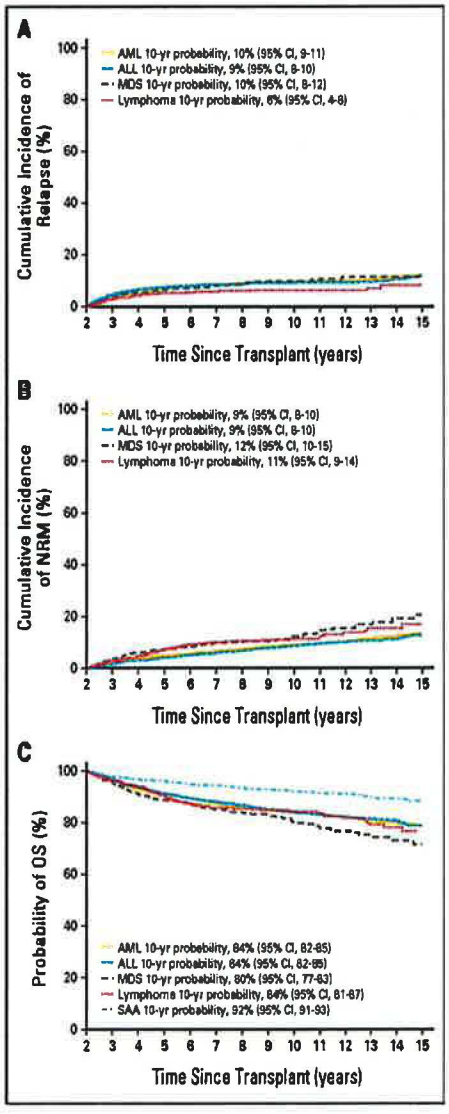

Figure 9: Outcome of 10,632 patients status post allogenic stem cell transplant including 2,171 patients with severe aplastic anemia [84]

Long-term Complications

Clonal evolution will occur long-term in about 15% of patients.[67] Most patients will manifest this by developing aneuploidy on bone marrow cytogenetics, have a recurrence of their pancytopenia, or evolve to myelodysplastic syndrome/acute leukemia.[66] Almost all patients with clonal evolution have telomere lengths in the lowest quartile at diagnosis.[36]

Other Human Telomere Diseases

Pulmonary Fibrosis

20% of patients with dyskeratosis congenita develop pulmonary fibrosis and studies of mutant pedigrees show a much higher incidence of this disorder than expected. Pulmonary disease is the second most common cause of death in patients with

dyskeratosis congenita.[23,85,86] In patients with the autosomal dominant form of dyskeratosis congenita, subsequent generations can exhibit a phenomenon termed anticipation where the pulmonary manifestations present earlier and more severely with each successive generation.[87-89] This would suggest that it is the length of the telomere and not the mutation that determines the severity of the pulmonary disease.

It is now known that up to 15% of patients with familial idiopathic pulmonary fibrosis have telomerase mutations.[90,91] Environmental factors likely play a large role in determining which patients will develop clinical manifestations of pulmonary fibrosis. Cigarette smoking is associated with a dose-dependent shortening of telomeres in smokers who develop COPD.[92]

Cirrhosis

Approximately 5% of patients with cirrhosis have no demonstrable risk factor such as viral hepatitis, excessive alcohol intake, or fatty liver disease.[93] In 1995, a relationship was demonstrated between telomere shortening and cirrhosis.[94]

Other Disorders

More and more disorders are being linked with short telomeres including atherosclerotic heart disease,[95,96] obesity,[97] osteoporosis,[98] and even psychological stress.[99] Some studies suggest that lifestyle modification may be able to lengthen telomeres.[100]

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