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Updates in acquired aplastic anemia: Can we do more for our patients?

Ana-Maria Moldovianu, Anca Popp, Zsofia Varady, Alina Tanase, Alexandra Marculescu, Camelia Dobrea, Didona Vasilache, Cerasela Jordan, Radu Niculescu, Daniel Coriu

Centre of Hematology and Bone Marrow Transplant, Fundeni Clinical Institute, Bucharest, Romania

Abstract:

The purpose of this work is to present the results of allogeneic stem cell transplantation as therapy for patients diagnosed with acquired aplastic anemia in the Department of Bone Marrow Transplantation of Fundeni Clinical Institute and to elaborate an algorithm of treatment in aplastic anemia starting with the observations obtained from our clinical practice and following the European treatment guidelines in this group of patients.

Aplastic Anemia (AA) is a rare hematological disease characterized by pancytopenia and a hypocellular bone marrow. The paradigm of bone marrow failure syndromes, aplastic anemia is a diagnosis of exclusion despite the precision of its diagnosis criteria. Although AA is not a malignant disease, but an autoimmune disorder, the grave consequences of pancytopenia and clonal transformation into acute leukemia make it a potentially fatal condition.

The management of AA patients is challenging and necessitates a very well established treatment plan from the diagnosis.

We present the treatment algorithm for AA patients with recommendations based on both recent guidelines in the field and on our experience treating AA patients with allogeneic stem cell transplant. Therapeutic procedure algorithm comprises different approaches for different patient populations, age categories and availability of immunosuppression therapy or different types of donors.

According to the recent EBMT recommendations the treatment of choice for young patients (younger than 40 years) who have a matched sibling donor is hematopoietic stem cell transplantation (HSCT). For those patients who don't have a matched sibling donor or are not candidates for HSCT

due to older age, the immunosuppression with ATG and cyclosporine is an efficient treatment. The supportive care has an important role and the patients with aplastic anemia should be managed by a multidisciplinary team. For patients older than 40 years, the choice between immunosuppressive therapy (IST) and upfront transplant with HLA identical sibling donor remains a key question. However, the standard approaches for this category of patients is front line immunosuppression with ATG and cyclosporine and if they become refractory to at least one course of IST the allogeneic stem cell transplant using fludarabine-based conditioning is the second-line treatment option.

In our institution there were eleven AA patients treated with allogeneic stem cell transplantation from 2009 till 2015. They were all young patients with age between 19 and 42 years old and all had severe acquired aplastic anemia with transfusion dependence. Six cases were transplanted from a matched sibling donor and five patients had undergone an unrelated matched donor transplant. The allogeneic HSCT procedure was done both as front line therapy in the case of three patients and as second treatment choice in the rest of eight patients. Four patients died, three of them due to transplant related toxicity and one patient experienced severe autoimmune reaction with transfusion inefficacy complicated with intracerebral haemorrhage at four months from transplant.

In our opinion the most challenging aspect in treating AA patients is choosing the best treatment option taking into account the patient age and performance status, the severity of the disease and the availability of a donor for allogeneic HSCT.

Although the treatment strategy must be individualized in every patient case, it is necessary to make a standardization of treatment procedures in AA and to follow the evidence based recommendations available in the management of this rare disease.

Keywords: aplastic anemia, bone marrow transplant, bone marrow failure, allogeneic stem cell transplantation, anti-thymocyte globulin, cyclosporine A.

Corresponding author

Ana-Maria Moldovianu, Department of Hematology, Fundeni Clinical Institute, Sos. Fundeni nr. 258, sector 2, Bucharest, Romania, phone+40745538575, e-mail: ana_maria_msg@yahoo.com

1. Definition

Aplastic anemia is defined as pancytopenia with a hypocellular bone marrow. Although bone marrow function is always diminished affecting all hematopoietic lineages, levels of blood

cells may not be depressed uniformly and different degrees of cytopenia can be observed. There are standard definition criteria of AA with different severity degrees. Severe AA is defined as BM cellularity $< 25\%$ and at least two of three criteria consisting in neutrophil count $< 0.5 \times 10^9/L$, platelet count $< 20 \times 10^9/L$, reticulocytes $< 20 \times 10^9/L$ on manual count or $< 60 \times 10^9/L$ on automated analyser. The very severe form of AA is when the criteria for severe AA are met and absolute neutrophil count is under $0.2 \times 10^9/L$. AA is classified as non-severe or moderate when the bone marrow is hypocellular but the peripheral blood values do not meet the severe criteria. This classification is of prognostic relevance and has an influence on therapeutic approaches.

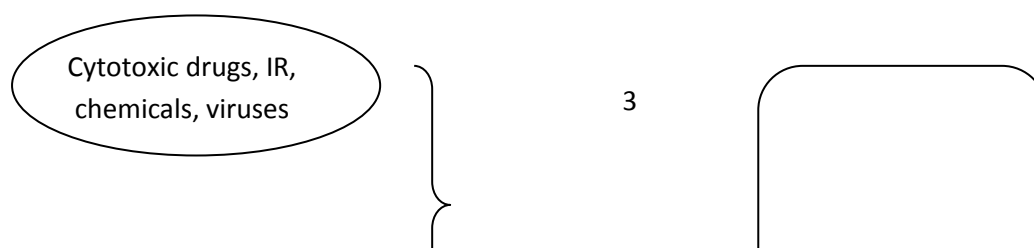
Aplastic anemia can occur as a primary hematologic disorder most often idiopathic, or apparently result from various causes like physical and chemical toxins, drugs or viruses that can act directly or indirectly by an immune mechanism. In about 70% of cases, aplastic anemia is thought to be caused by autoimmune-mediated suppression of the bone marrow by T cells releasing tumor necrosis factor and interferon- γ causing apoptosis of CD34+ progenitor cells.

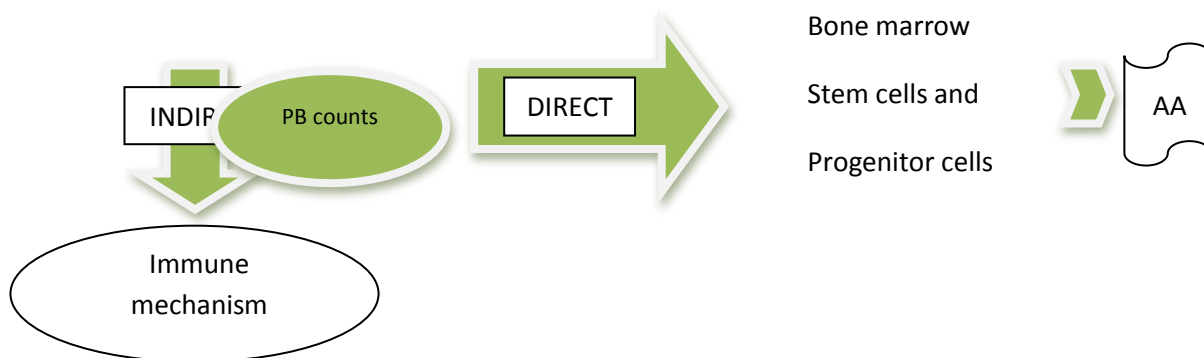
2. Classification

Aplastic anemia also can be classified based on presumed etiology in two forms: acquired and inherited. More than 80% of cases are idiopathic, 20% are drug-induced, and the post-infectious form is found in less than 5% of the cases. The hereditary form with initial manifestation at adult age (late onset hereditary bone marrow failure syndromes) like congenital dyskeratosis or related telomeropathies is found in less than 1% of the AA cases. This classification has not been proved to have a prognostic or therapeutic relevance except for the drug-induced AA in which the drugs in question must be stopped and re-exposure avoided.

All congenital bone marrow failure syndromes as well as radiation or chemotherapy induced aplasia are excluded from the acquired AA.

Figure 1. The etiologic mechanisms of AA





3. Epidemiology

According to International Aplastic Anemia and Agranulocytosis Study (IAAAS) conducted in Europe and Israel from 1980 to 1984, overall annual incidence of AA is 2 cases per 1 million people. There are no racial or sex differences in the occurrence of AA. The age distribution shows two peaks, one between 10 and 25 years, and a second among the over 60 year-olds.

4. Diagnosis of acquired AA

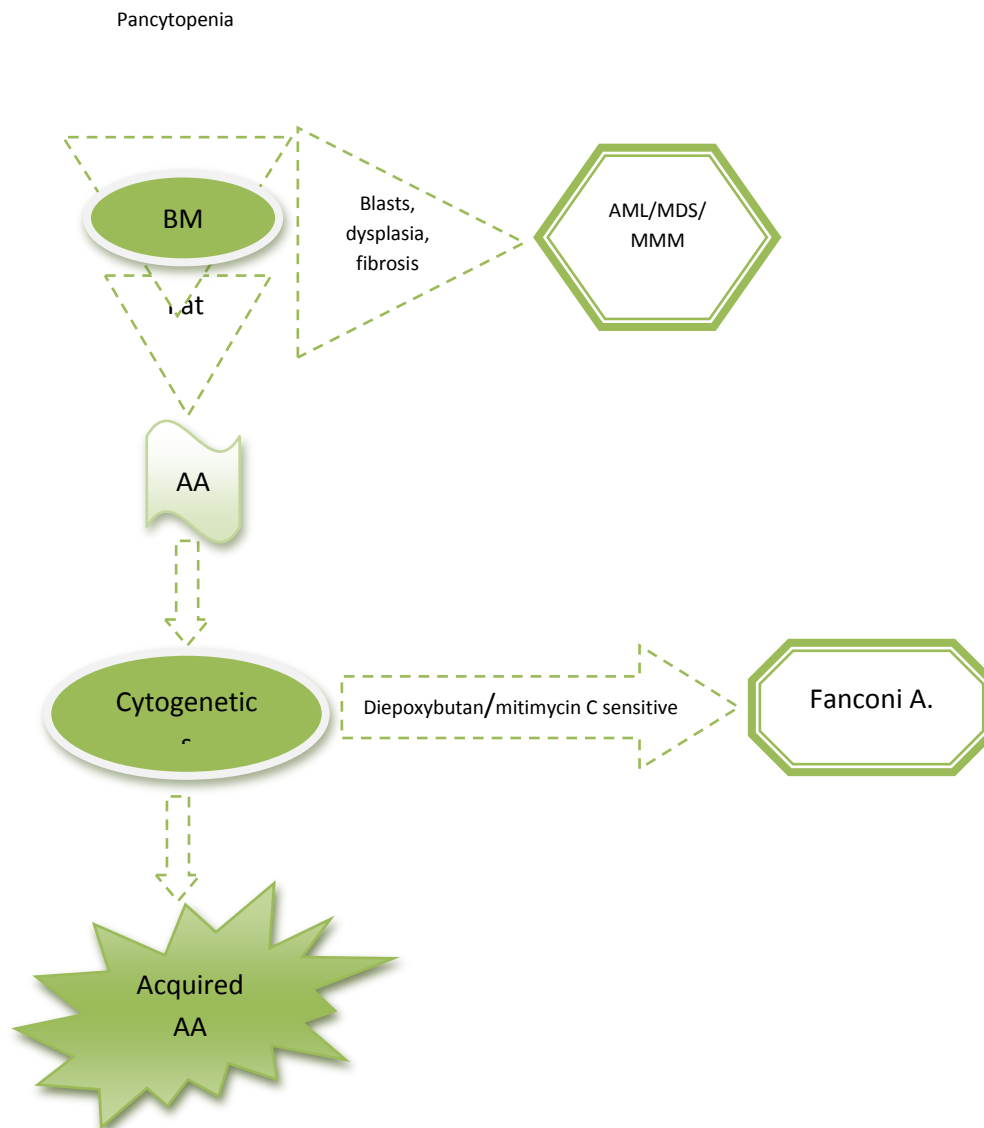
The diagnosis algorithm of AA patients is presented in **figure 2. Diagnostic algorithm in AA.**

The most important steps in making diagnosis of AA are:

- Confirm the suspicion of AA and exclude other bone marrow failure diseases
- Define the severity of the disease
- Characterize the disease.

The criteria for diagnosis of AA are bi- or pancytopenia on complete blood count and bone marrow aplasia or hypoplasia. Anemia is often normocytic, occasionally moderately macrocytic. Leukocytopenia is resulting from granulocytopenia and monocytopenia, often there are no immature precursors cells and the giant platelets are absent in blood smears. Bone marrow aspirate and biopsy are mandatory and the length of biopsy fragment must be at least 15 mm. The neoplastic cells and fibrosis must be absent. Often there is a focal decrease in medullary density.

Figure 2. Diagnostic algorithm in AA



In children and young adults, acquired AA should be distinguished from the inherited forms of bone marrow failure such as Fanconi's anemia as the differentiation has therapeutic implications. Patients with Fanconi's anemia often have physical anomalies, but the distinction depends on the laboratory finding of abnormal chromosome fragility seen readily in metaphase preparations of peripheral blood lymphocytes cultured with phytohemagglutinin. Chromosomal breakage is strikingly enhanced compared with controls if clastogenic agents, such as diepoxybutane, are added to the culture.

However, in older patients, the major differential diagnosis is between AA and hypoplastic myelodysplastic syndrome which sometimes may be one of the most difficult diagnostic issues. The main differences between these two entities are the absence of dysplasia, splenomegaly, blast cells, marrow fibrosis and cytogenetic anomalies in AA which

may be present in MDS; CD 34 positive cells are absent in AA, but in MDS may be normal or increased, and myelopoiesis and megakariopoiesis usually are absent in AA, but possible in hypoplastic MDS.

The severity of AA is exclusively based on peripheral blood parameters and not on bone marrow cellularity. The criteria defining the severity of the disease were presented in the definition paragraph.

Regarding the characterization of AA, there are some important interrelations between AA and other conditions that may have impact on disease outcome and prognostic and therapeutic relevance.

Acquired AA and Paroxysmal Nocturnal Hemoglobinuria (PNH) have a close interrelationship. Patients diagnosed with AA often present a PNH clone. Also, patients with PNH can develop AA in the course of their disease. In the AA patients most clones are small and they do not have symptoms related to PNH. However, in some patients the PNH clone can increase in the evolution of AA and the PNH characteristic symptoms and complications become the problem to deal with. There are some data predicting a better response to immunosuppressive therapy in AA patients with significant clones of PNH.

Other association observed in 5-10% of aplastic anemia cases is with seronegative hepatitis. It is characterized by self-limited liver inflammation, elevated liver enzymes often severe, negative viral serology for hepatitis A, B or C. The post-hepatitis AA typically occurs in young, healthy males and it seems to have a poorer prognosis than idiopathic AA, with early estimates of mortality of 90% at one year, and a history of hepatitis in AA has been considered an indication for early BMT. Patients with posthepatitis AA can successfully undergo BMT without an increased risk of veno-occlusive disease. Patients with hepatitis-associated AA have markers of immune system activation and respond well to intensive immunosuppressive therapy.

Pregnancy is common in the age groups most susceptible to BM failure, and in many cases, its association is probably only coincidental. The true frequency of AA in pregnancy is unknown, but from the number of cases reported, it appears rare, although bone marrow hypoplasia may be relatively common during pregnancy. Survival rates for AA in pregnancy have been relatively high for the mother and baby, with the most pregnancies being successful. The published data are insufficient to guide the management of pregnant women with AA. A woman who desires a child can be maintained with transfusions, as hemorrhage is the most common cause of death from AA during pregnancy.

In a large multicenter study of the Severe AA Working Party of the EBMT the association between aplastic anemia and an autoimmune disease was reported in 50 of 1251 AA patients, especially in older patients. Aplastic anemia is a component of the collagen vascular syndrome called eosinophilic fasciitis. This severe, scleroderma-like disease is characterized by fibrosis of subcutaneous and fascial tissue, localized skin induration, eosinophilia, hypergammaglobulinemia, and an elevated erythrocyte sedimentation rate. The rheumatologic symptoms of fasciitis respond to corticosteroids, but the associated AA has a very poor prognosis. More rarely, AA has complicated systemic lupus erythematosus (SLE) and rheumatoid arthritis, but in many cases, the role of concomitant drug therapy is intricately. Rarely, AA can accompany Sjogren syndrome, multiple sclerosis, and immune thyroid disease. AA occasionally occurs in individuals with hypogammaglobulinemia or congenital immunodeficiency syndrome, thymoma or thymic hyperplasia.

5. Differential diagnosis and diagnosis by exclusion

The differential diagnosis for acquired aplastic anemia must include: hypoplastic acute leukemia, myelodysplastic syndrome (hypoplastic form), hairy cell leukemia and other lymphomas, bone marrow infiltration by solid tumors, osteomyelofibrosis, hypersplenism, severe megaloblastic anemia, anorexia nervosa, systemic lupus erythematosus, paroxysmal nocturnal hemoglobinuria, Fanconi anemia, congenital dyskeratosis, Shwachman-Diamond syndrome, isolated aplastic anemia (“pure red cell aplasia”), aplasia after chemo- or radiation therapy.

6. Diagnostic workup for AA

- Detailed medical drug therapy history
- Clinical examination paying attention to clinical signs relevant for cytopenic complications or differential diagnosis: infection, bleeding, lymphadenopathy, hepatomegaly, splenomegaly, jaundice, nail dystrophies, leukoplakias, pigment or skeletal or dental anomalies
- Complete blood counts including differential and reticulocytes
- Bone marrow biopsy and aspirate, cytogenetic studies
- LDH, haptoglobin, hemosiderin in the urine looking for hemolysis
- Coagulation tests
- Reactive C Protein test
- Blood total protein, serum protein electrophoresis and immunoglobulins
- GOT, GPT, creatinine, urea, uric acid, glucose
- Ferritin

- Vitamin B12, folic acid
- Immunological tests like ANA, anti-DNA antibodies
- Blood group, Coombs tests
- Chest X-ray, abdominal sonography
- EBV, CMV, hepatitis A+B+C, HIV, Parvovirus B19
- Flow cytometric analysis of GPI-anchored proteins on granulocytes and erythrocytes
- Telomeric length measurement by means of the Flow-Fish method (if shortened telomeres then mutation analysis for TERT, TERC, TIN2, and further components of telomerase complex if possible) in the case of adolescents and young adults if congenital bone marrow failure syndromes are suspected
- For differential diagnosis with Fanconianemia: chromosome fragmentation test, mutation analysis of the Fanconianemia genes
- HLA class I and II typing for allogeneic HSCT transplant candidates

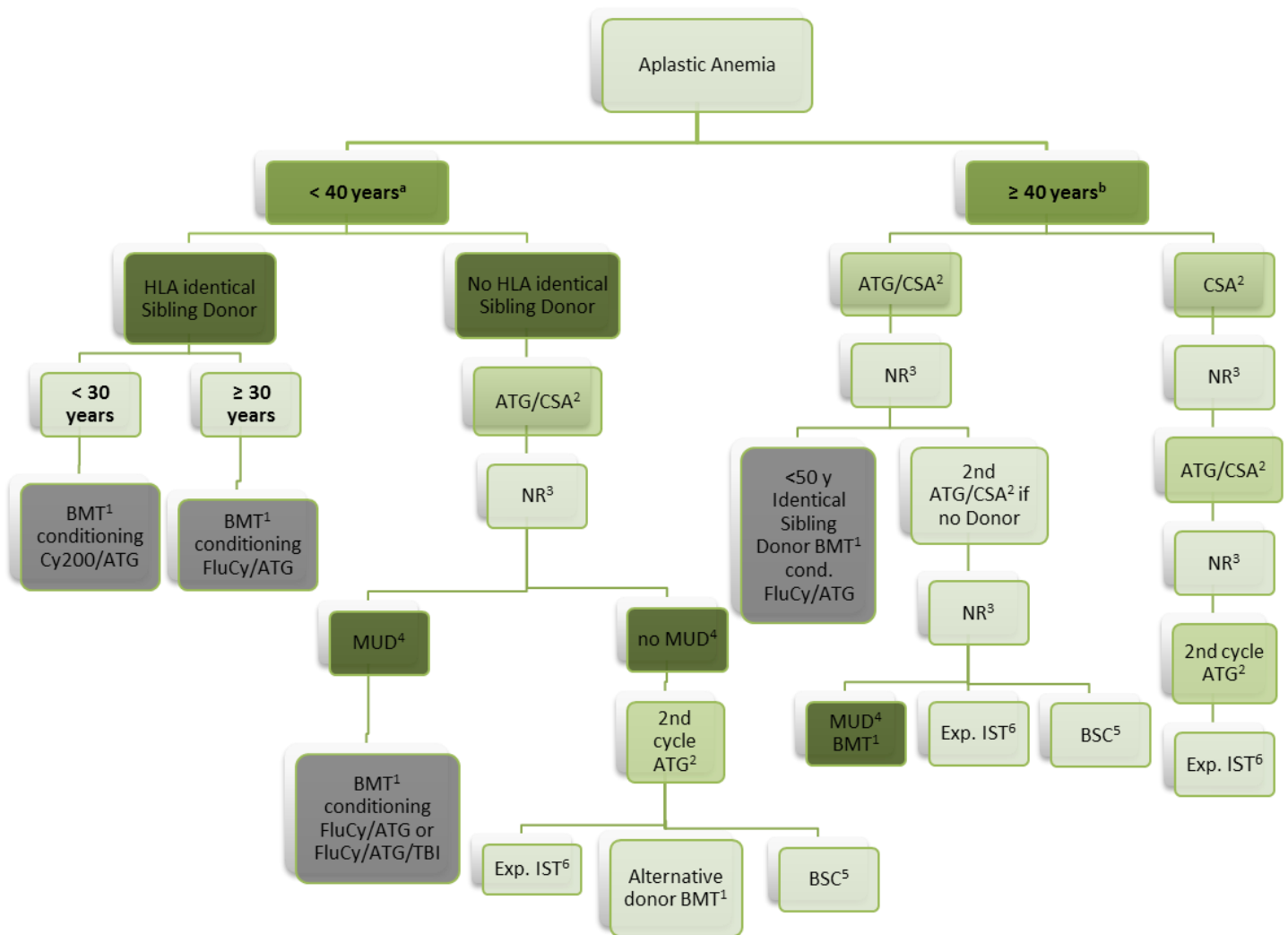
7. Therapy

a. Indication

The management for AA patients should be based on a therapy plan promptly made once the diagnosis has been confirmed. Treatment options must be identified and chosen with alacrity. The choice of therapy depends on the age of the patient, the severity of the disease, and the degree of the HLA-identity in a potential related or unrelated bone marrow donor (**Figure 3. The therapeutic algorithm for adult patients with Aplastic Anemia**).

The interval between diagnosis and therapy, as studies have shown in cases of bone marrow transplantations, has a significant impact on the outcome. A therapy that is intended to cure will be indicated in three situations: symptomatic disease, severe disease or high risk disease. The patients with severe and very severe form of AA according to definition and the cases with non-severe AA but with severe cytopenia of at least one cell line which requires regular transfusions or with an increased risk for infections or bleeding invariably need definitive treatment. Also, progression of non-severe AA to severe AA is an indication for definitive therapy. Other situations are to be individually evaluated taking into consideration the course of the disease.

Figure 3. The therapeutic algorithm for adult patients with acquired Aplastic Anemia



Legend:

^aModulate according to disease severity, favour transplant if very severe disease

^bIn the very elderly treatment may be limited to Cyclosporine A

¹BMT – bone marrow transplant, Cy200/ATG-cyclophosphamide 200 mg/kgc and ATG; FluCy/ATG-Fludarabine 120mg/m², low-dose Cyclophosphamide 120mg/kgc and ATG; TBI-total body irradiation

²ATG – antithymocyte globulin, CSA-cyclosporine A, IST-immunosuppressive therapy

³NR – non - responder

⁴MUD – Matched Unrelated Donor

⁵BSC – Best supportive care including transfusional support, trial of androgens

⁶Exp. IST – experimental immunosuppressive therapy (Alemtuzumab, high dose cyclophosphamide)

The current indications for allogeneic HSCT in patients diagnosed with acquired AA according to the last report of the European Society for Blood and Marrow Transplantation published in 2015 regarding the current practice and indications for HSCT for hematological diseases, solid tumors and immune disorders in Europe are the following:

- For newly diagnosed AA patients allogeneic HSCT from sibling donor is a standard procedure, allogeneic HSCT from well matched unrelated donor is also standard of care in children and may be a clinical option in adult patients. Alternative donor allogeneic HSCT is generally not recommended as front-line treatment in AA.
- For relapsed/refractory AA patients standard of care includes sibling allogeneic HSCT or well matched unrelated donor allogeneic HSCT in the absence of a suitable family donor. Alternative allogeneic HSCT remains a clinical option which can be carried after careful assessment of risks and benefits.
- Autologous stem cell transplantation has no role in the management of AA. Well-matched unrelated donor means a 10/10, 8/8 or 9/10 (if mismatch in DQB1) allele matched donor. Alternative donors denote multiple mismatched unrelated donor, cord blood, and haploidentical transplants.

b. Allogeneic Stem Cell Transplantation

Allogeneic Stem Cell Transplantation from an HLA-matched Sibling Donor

Since 2009, in our Hematology and Bone Marrow Transplantation Center, six patients diagnosed with severe acquired AA were treated with bone marrow transplant (BMT) from identical sibling donor. Three patients received this therapy as frontline treatment, the other three as a secondary therapy after failing combination immunosuppressive therapy (IST) with ATG and cyclosporine A (CsA). The characteristics of the patients are presented in **Table 1. Characteristics of patients treated with allogeneic HSCT from HLA identical sibling donor.**

The median age at the time of BMT in this group of patients was 29 years (19-37 years). The conditioning regimen used was Cy200/ATG and the source of stem cells was the peripheral blood (PBSC) in all the six cases. Graft versus host disease (GvHD) prophylaxis was done with CsA and short course methotrexate (MTX). After 4-year median follow up, five of six patients are surviving.

Unfortunately, one patient died day + 16 after BMT due to severe infection. In the group of second-line therapy transplanted patients there was one case of graft failure at one year after BMT. The patient successfully received a second stem cell transplant. The second BMT conditioning regimen used in this case included fludarabine and total body irradiation (TBI). He had increasingly mixed chimerism at the time of graft rejection. The posttransplant immunosuppressive therapy with cyclosporine A was continued for about one year with slow dose tapering in all the cases. Remarkably, in both groups of patients there was no acute or chronic grade III/ IV GvHD.

Table 1. Characteristics of patients treated with allogeneic HSCT from HLA identical sibling donor

Characteristics	Patients with sibling matched donor BMT					
	First-line therapy			Second-line therapy (Relapsed/refractory pts)		
Age	29 y	37 y	37 y	19 y	26 y	22 y
Diagnosis	SAA	SAA	SAA	SAA	SAA	SAA
Conditioning	Cy200/ATG	Cy200/ATG	Cy200/ATG	Cy200/ATG	Cy200/ATG	Cy200/ATG
Blood group matching	Yes	Yes	Yes	Yes	Yes	Yes
GvHD	No	NA	No	No	No	No
Chimerism	100%	NA	100%	100%	Mixt donor	100%
Graft failure	No	NA	No	No	Yes, 1 y after BMT	No
Stem cell S.	PB	PB	PB	PB	PB	PB
Time DTx	3 months	2 months	5 months	1,5 years	3,5 years	1,5 years
Status	Alive	Deceased Day +16 after BMT	Alive	Alive	Alive	Alive
Complications	No	Infection, sepsis, MSOF	Pretransplant Siphilis infection-AB prophylaxis	No	2 nd SCT Flu+TBI, 100% chimerism	No

Legend:

BMT – bone marrow transplant, Cy200/ATG-cyclophosphamide 200 mg/kgc and ATG; FluCy/ATG- Fludarabine 120mg/m², low-dose Cyclophosphamide 120mg/kgc and ATG; TBI-total body irradiation
 ATG – antithymocyte globulin, CSA-cyclosporine A, IST-immunosuppressive therapy
 SCT – stem cell transplant
 AB – antibiotic therapy
 PB – peripheral blood cells
 DTx – time between diagnosis and transplantation
 MSOF – multiple system organ failure
 GvHD – graft versus host disease

Discussion:

Allogeneic bone marrow transplantation from an HLA-identical sibling donor is the standard of care in the following situations: as front-line therapy in cases of severe or very severe AA and age < 40 years and as second line therapy in cases of severe AA and age < 50 years after the failure of at

least one cycle of immunosuppressive combination therapy with ATG and cyclosporine A (**Figure 3. The therapeutic algorithm for adult patients with Aplastic Anemia**).

Transplantation for AA from an HLA-identical sibling donor has improved considerably over the years, with a 75% to 80% chance of long-term cure. A recent report from the EBMT of over 1500 patients confirmed that predictors of survival following BMT included matched sibling donor, age of less than 16 years, early transplant (time from diagnosis to transplant of less than 83 days) and a non-radiation conditioning regimen.

In our opinion, the most important aspects regarding best frontline therapy in AA patients are the age of the patient, the severity of the disease and the availability of an HLA-identical sibling donor. The cutoff age of 40 years for frontline BMT is controversial. The latest studies showed that the outcome of patients in the ranges of 20 to 30 years, 30 to 40 years and 40 to 50 years tend to be similar. However, even if the actual tendency is to shift the upper age limit to 50, we consider that upper limit of “young age” should be probably situated around age 30-35 and the disease severity and performance status should be taken into discussion. Obviously, response to allogeneic HSCT may be more rapid as compared to IST and may be preferable in a situation of an infected or transfusion refractory patient. Also, one study demonstrated that the outcome in patients undergoing transplantation after failing IST is worse than undergoing transplantation upfront.

However, retrospective analysis of our AA patients grafted from sibling matched donor showed that at 4 years median follow-up the mortality in both group of patients- first line BMT and second line BMT, was related only to transplant procedure (infection precociously occurred after BMT). The meaning is that transplant related toxicity is a real concern.

In younger patients with AA, the standard conditioning proposed by the Working Party on SAA is cyclophosphamide 50 mg/kg x 4 + ATG (Cy200+ ATG). This regimen is nonmyeloablative and highly immunosuppressive to prevent graft rejection and GvHD. Because of the unsatisfactory results and in order to reduce transplant-related toxicity and improve survival of patients older than 30 years with sibling transplantation, the use of less cytotoxic but more immunosuppressive regimens was explored in several studies with encouraging results. Modified regimens included low dose cyclophosphamide ($300 \text{ mg/m}^2 \times 4$) in combination with fludarabine ($30 \text{ mg/m}^2 \times 4$) and ATG. Also, according to the EBMT SAA working party retrospective analysis of 30 patients older than 30 years receiving reduced-intensity conditioning BMT, there was a higher probability of age-adjusted overall survival than the control group.

Based on these results, we consider that treating AA patients older than 30 years with up front allogeneic stem cell transplant from sibling HLA-matched donor using fludarabine – based conditioning may be a better option than with standard high dose cyclophosphamide conditioning

HSCT. This new conditioning protocol is being evaluated in a study of the EBMT Aplastic Anemia Working Party. Also, in this combination of fludarabine with low dose cyclophosphamide, alemtuzumab may be an alternative to ATG.

GvHD prophylaxis consists of cyclosporine combined with short course of methotrexate (5 mg/m² on day +1, +3, +6) and is the standard regimen associated with significant advantage in survival compared to CsA alone. It is recommended to administer therapeutic doses of cyclosporine over a period of at least nine months and then gradually reduce and stop therapy at least over a period of three months on the surveillance of the chimerism status. The risk of graft failure is increased in the case of progressive increase of host cells > 5%. The lowest risk of graft failure is achieved when there is full donor chimerism or stable mixed chimerism by one year after BMT, but no more than 5 % host.

Stem cell source should be the bone marrow as retrospective studies (observational study by Center for International Bone and Marrow Transplant Research and EBMT) have showed that peripheral blood stem cells grafts are associated with higher rates of both chronic GvHD development and mortality than the transplantation with bone marrow stem cells. In a retrospective analysis of EBMT the authors concluded that bone marrow grafts are preferable especially in young patients undergoing HLA-matched sibling donor transplantation for SAA, as there was an approximately 10% survival advantage between the two types of transplantation favouring the bone marrow stem cells (the 5-year overall survival was 85% after transplantation with bone marrow cells and only 73% after peripheral stem cells grafting).

In our group of patients the source of stem cells used in all the six grafts was only PBSC and as we can see there is no significant GvHD, acute or chronic.

Allogeneic Stem Cell Transplantation from an Unrelated Donor or Alternative Donor

In our medical center, in the same period of time as mentioned above, five patients diagnosed with severe AA and without a sibling donor received allogeneic stem cell transplant from unrelated HLA compatible donor. The characteristics of the patients are presented in **Table 2. Characteristics of patients treated with allogeneic HSCT from HLA Identical Unrelated Donor.**

All unrelated transplant cases were with 10/10 allele matched donor. In all the cases unrelated transplantation was done as secondary therapy after failing immunosuppressive therapy (IST) consisting of combination with ATG and CsA in the case of two patients and respectively monotherapy with CsA in three cases. The median age at the time of BMT was 26 years (21-42 years). The median time between diagnosis and BMT was 10 months (5 months-66 months). Conditioning regimen used included fludarabine 30 mg/m² x 4, low dose cyclophosphamide 30 mg/kg x 4 and ATG 10 mg/kg x 4. The source of stem cells was bone marrow in one case and the

peripheral blood stem cells were used in the rest of the cases. Three patients died, one on day -1, one on day +9 and one at four months after BMT. The probable causes of death were toxicity related to conditioning regimen, especially to ATG, in two of the cases and an autoimmune reaction occurred after approximately four months posttransplantation causing transfusion refractoriness and severe bleeding in the case of one patient. There was no significant acute or chronic GvHD and no graft rejection, all the patients having stable complete donor chimerism. The GvHD prophylaxis was cyclosporine and mini dose methotrexate. The complications after BMT developed by our patients included opportunistic infections like CMV reactivation in two cases and hepato-splenic tuberculosis in one patient. With adequate treatment these complications were manageable and patients were cured.

Discussion

Standard indication for unrelated stem cell transplantation is as secondary therapy in the case of severe AA or very severe AA and age ≤ 40 years after the failure of at least one cycle of immunosuppressive combination therapy with ATG and CsA and absence of suitable sibling donor (**Figure 3. The therapeutic algorithm for adult patients with Aplastic Anemia**).

Unrelated matched donor stem cell transplant may be taken into discussion in patients > 40 years of age and with a good performance status if other treatments failed. As primary therapy, at present, there is no consensus. In young patients with very severe AA, unrelated BMT may be an option if a donor with a 10/10 match is available.

As in the case of sibling donor BMT, the patient age and performance status and the severity degree of the disease come into question for an unrelated transplantation. The search for an unrelated donor should be initiated at an early stage. Suitable donor in AA is considered only when there is a high resolution HLA match 10/10. There are no recommendations about when to accept mismatched unrelated donors.

More than 70% of patients will do not have an HLA-matched sibling donor. Alternative potential donors include relatives who are phenotypically matched or partially matched and HLA phenotypically matched but unrelated volunteers.

Table 2. Characteristics of patients treated with allogeneic HSCT from HLA Identical Unrelated Donor

Characteristics	Patients with Unrelated Matched Donor BMT				
	as				
	Second-line therapy (relapsed/refractory patients)				
Age	42 y	21 y	26 y	31 y	23 y
Diagnosis	SAA	SAA	SAA	SAA	SAA
First-line IST	CsA	ATG(1)+CsA	ATG(1)+CsA	CsA	CsA
Conditioning	FluCy/ATG	FluCy/ATG	FluCy/ATG	FluCy/ATG	FluCy/ATG
Blood group matching	NO	Yes	Yes	Yes	Yes
GvHD	NA	No	No	No	NA
Chimerism	NA	100%	100%	100%	NA
Graft failure	NA	No	No	No	NA
Stem cell S.	PB	PB	MO	PB	PB
Time DTx	6 months	10 months	6 months	5,5 years	8 months
Status	Deceased Day +9 after BMT	Deceased Day +123 after BMT	Alive	Alive	Deceased Day -1
Complications	Toxicity related to ATG	Autoimmune reaction, transfusion refractoriness, brain hemorrhage	Leg cellulitis	CMV reactivation; Hepatic and splenic tuberculosis	Toxicity related to ATG

Legend:

BMT – bone marrow transplant, FluCy/ATG-Fludarabine 120mg/m², low-dose Cyclophosphamide 120mg/kg and ATG;

ATG – antithymocyte globulin, CSA-cyclosporine A, IST-immunosuppressive therapy

PB – peripheral blood cells

DTx – time between diagnosis and transplantation

GvHD – graft versus host disease

NA – not applicable

Although phenotypically identical family donors are occasionally available, mismatched family members and matched but unrelated donors represent a much larger pool. Unfortunately, unrelated donors and mismatched transplants have almost twice the transplant-related mortality and risk of GvHD as matched sibling donor transplants. Also, graft rejection is a big obstacle for unrelated transplants. Historically, in the large European experience, for phenotypically identical family matches, the actuarial survival rate was 45%, for patients with a single-locus mismatch, it was 25%, and for those with two to three loci mismatched, the survival rate was 11%.

According to an early EBMT report, the best results with unrelated or mismatched transplantation are seen in patients under the age of 21 years with disease duration of less than one year. Also, the International Bone Marrow Transplant Registry reported on a group of 318 alternative donor transplants in patients with SAA between 1988 and 1998, a 20% probability of graft failure and a survival probability at 5 years less than 40%. Most patients were young, heavily transfused and of poor performance status.

The Fred Hutchinson Cancer Research Center (Seattle, WA, USA) reported on the results of unrelated allogeneic BMT in SAA after conditioning with low-dose TBI, high-dose cyclophosphamide and ATG. The median age was 19 years, and with a median follow-up of 7 years, 61% of HLA-identical and 40% of HLA mismatched transplant recipients survived the procedure; however, more than 70% of patients acquired acute GvHD and over 50% developed chronic GvHD.

A recent meta-analysis of 18 heterogeneous trials evaluating the outcomes for patients who received unrelated donor transplants after failure to respond to IST, suggests that a good performance status and detailed HLA-matching contribute to improved survival.

A review of data from the EBMT analyzed 498 patients transplanted during 1990–2005. Only the year of BMT was associated with increased survival. Survival at 5 years increased from 38% before 1998 to 57% after 1998. Also after 1998, there was less graft failure, less acute and chronic GvHD. The authors suggest that these improvements in outcomes are due to better donor matching.

The outcome of unrelated donor transplants for SAA patients has improved not only due to better selection of HLA matched donors but also to significant changes in the conditioning regimen. A recent analysis from the EBMT-SAA working party retrospectively reviewed the outcome of 100 patients treated with alternative donor transplant as secondary therapy after the failure of IST. All patients received conditioning with a combination of fludarabine 30 mg/m²x4, cyclophosphamide 300 mg/m²x4 and ATG (FCA) with or without low dose (2 Gy) TBI. The actuarial 5-year survival was 73% for the group that received FCA and 79% for the group given the conditioning regimen including TBI. The most significant predictor of survival was the interval between diagnosis and transplantation, with 5-year survival rates of 87 and 55% for patients grafted within 2 years of

diagnosis and more than 2 years after diagnosis, respectively. The overall cumulative incidence of acute GvHD grades II–IV and III–IV was 18% and 7%, respectively, with no difference between the two regimens. Chronic GvHD was recorded in 27% of the FCA group and 50% of the FCA-TBI group. This study confirmed that survival of patients with SAA treated with unrelated donor transplant has almost doubled in the past decade.

Even more than in standard sibling transplants, age is a crucial risk factor in unrelated transplants and probably more important than the level of match, conditioning regimen, or use of T-cell depletion.

EBMT recommends that treatment with alternative donor transplant should be adapted. In children and young adults (between 20 and 30 years) without a matched sibling donor, an unrelated donor should be started at diagnosis and transplantation should be considered after one course of IST had failed, in the presence of a suitable donor (10/10 matched donor). At the best centers, survival rates now are almost as good as with sibling donors. Adults do less well, primarily because of transplant-related mortality from the intensive conditioning regimen and so for adults over the age of 30 there are no clear indications and enrollment in a prospective trial is advised. Alternative donor transplant may be an option for second line treatment after failure of one or two courses of IST for this patient category depending on patient performance status and comorbidities and disease severity.

Current protocols within EBMT include two dose-reduced conditioning regimens which are modified relative to sibling donor transplantations: a radiation free regimen with a combination of low-dose cyclophosphamide 300 mg/m²x4, fludarabine 30 mg/m²x4 and ATG x4 days (FCA) or alemtuzumab for patients under the age of 14 years and an adjusted regimen with addition of 2 Gy total body irradiation (TBI) to Flu-Cy and administration of half of the dose of ATG (2 days instead of 4 days) in patients above the age of 14 years up to the age of 55 years. The reason to add TBI in adults was based on a high rejection rate with FCA in patients over the age of 14.

An alternative approach consists in conventional cyclophosphamide conditioning Cy200 mg/kg with ATG and low-dosed TBI (2Gy) or the combination of reduced dose of cyclophosphamide Cy120 mg/kg and 8 Gy TBI.

Donor selection includes suitable donors in the following order: 1. 10/10 allele matched unrelated donor (MUD), 2. One antigen mismatched UD, 3. matched or minimally mismatched cord blood or haploidentical donor.

Graft rejection and GvHD are the major complications of allogeneic transplantation in AA. Graft rejection is a major predictor of posttransplantation survival. The rate of graft rejection decreased with intensification of the immunosuppressive conditioning regimen from 15% to 4% in Europe and from 35% to between 10% in Seattle and has remained stable in the last decade. Graft

rejection can be caused by the pathophysiology of AA, a finding supported by the unexpectedly high proportion of failure in unprepared patients receiving syngeneic transplants and even in adequately preconditioned patients receiving syngeneic transplants. In a group of untransfused patients who received allogeneic stem cells, the incidence of graft rejection was 10%, indicating that AA patients may be particularly sensitive to alloimmunization. Nevertheless, the influence of the number of transfusions on graft rejection is relative, and modest number of blood donations (40 units in the International Bone Marrow Transplant Registry experience and less than 10 units of erythrocytes or 40 units of platelets in Seattle) did not greatly increase the risk of graft rejection.

Table 3. Clinical data of all AA patients treated with alloHSCT in our center

	Sibling matched donor BMT in 1st line therapy	Sibling matched donor BMT in refractory/relapsed patients	Unrelated matched donor BMT (only relapsed/refractory AA patients)
Patients	3	3	5
Age interval	29-37 y	19-26 y	21-42 y
Interval Dx-Tx	2-5 mos	1,5-3,5 y	6 mos-5,5 y
GvHD III-IV	0	0	0
Rejection	1	0	0
Surviving	2	3	2
Cause of death			
- Infection	1	-	-
- Conditioning toxicity	-	-	2
- Haemorrhage	-	-	1
- Graft failure	-	-	-
- GvHD	-	-	-

Despite progress, matched but unrelated transplantation in AA patients is associated with a high mortality rate as it was resulted also from our experience in our center. More than half of our patients grafted from unrelated matched donor (three of five patients) died (**Table 3. Clinical data of all**

AA patients treated with allo HSCT in our center). Refractory and high-risk patients are selected for this procedure and it is likely the poor results may be a consequence of this. However, alternative donor transplant is feasible. Alternative donor transplantation represents an option, especially for the young patient with very severe pancytopenia in whom immunosuppressive therapy has failed.

Late complication of bone marrow transplantation

Very late complications after transplantation include effects on growth and development, as well as on the function of endocrine, neurologic, and other organ systems. A high rate of secondary malignancies has been recorded after transplantation. For AA, among 320 patients who received transplantation in Seattle, four developed cancer, leading to a calculated risk seven times higher than for normal control participants. In a more recent update, 12 % of patients who survived more than two years after transplantation developed solid tumors. In an analysis of 700 transplanted patients with AA and Fanconi's anemia, the risk of developing a secondary malignancy was 14% at 20 years. The hazard of lymphoid malignancies decreased with the time after transplantation, but the risk of solid tumours progressively increased. In general, the rates of secondary malignancies after BMT for AA and other diseases are similar. Immune events such as GvHD, treatment with ATG or monoclonal antibodies, and irradiation have been related to the development of secondary malignancies. It is important to be mentioned that in AA patients there is a significant risk of late malignancy independent of transplantation therapy. The risk of malignancy in the large registry of EBMT was equivalent for patients who received immunosuppression and those who underwent transplantation. Compared with general European population, the relative risk of malignancy was calculated at 5.15 for AA patients treated with immunosuppression and at 6.67 for patients receiving transplants.

c. Immunosuppressive therapy (IST)

Combined or Intensive Immunosuppressive Therapy (ATG plus CsA)

Immunosuppressive therapy is an effective alternative treatment for patients who are not candidates for BMT (**Figure 3. The therapeutic algorithm for adult patients with AA**). Combination therapy with antithymocyte globulin (ATG) and Cyclosporine A (CsA) provides a response rate of 70-90%, and does significantly better than either agent alone with more complete response and better 5-year survival rates for responding patients.

Antithymocyte globulins are immunoglobulin preparations made from the sera of horses immunized against human thymocytes are the mainstays of current regimens. In Europe, the standard preparation of ATG has until recently been horse ATG (Lymphoglobuline) whereas in the United States was and is still used ATGAM. All large European Cooperative studies have been done using horse ATG. The European preparation has been withdrawn from the market and replaced by rabbit

ATG (Thymoglobuline) which was used for second or subsequent courses of IST (**Table 4. Different preparations of antithymocyte globulin**).

Response rates to rabbit ATG are anticipated to be similar with horse ATG based on response rates to second courses of IST, the same immunogen and a similar production method binding to similar epitopes. There have been only limited studies using rabbit ATG for the first-line IST for AA. The majority of the studies which examined rabbit ATG efficacy in the first line treatment for AA were non-randomized studies, which compared the results to historical controls and produced discrepant results. In contrast, a recently published randomized study comparing rabbit ATG with horse ATG showed that response rate and overall survival is significantly better with horse ATG. The response rate after three months was 62% with horse ATG as compared to only 33% in the rabbit group. Total survival rate of 85% with horse ATG was superior to only 55% survival rate after treatment with rabbit ATG. However, the change from horse ATG to rabbit ATG was done in Europe not on evidence-based reason, but due to lacking availability.

Table 4. Different Types of Antithymocyte Globulin[^]

ATG brand	Cells used for immunization	Animal species	Recommended dose
ATGAM	Human thymocytes	horse	40 mg/kgx4
Lymphoglobuline	Human thymocytes	horse	15 mg/kgx5
Thymoglobuline	Human thymocytes	rabbit	3.75 mg/kgx5
ATG-Fresenius	Jurkat T-ALL (acute lymphoblastic leukemia) cell line	rabbit	5 mg/kgx5

[^]Jakob R. Passweg – Aplastic Anemia: First-line Treatment by Immunossuppression and Sibling Marrow Transplantation, ASH, 2010

A hematologic response to ATG is usually seen within a few month of therapy. The average time to improvement in neutrophil number is one to two months. Transfusion independence occurs after two to three months after initiation of therapy. Continued improvement commonly occurs after three months; nevertheless, clinical status by three months is strongly correlated with long-term survival. Blood cell counts above the values from the definition criteria for severity, platelets of more

than 50.000/ μ L and reticulocytes are highly prognostic. In most recent studies reticulocyte count has been the best predictor of response and survival.

The major toxic effects of ATG are immediate allergic phenomena, serum sickness, and transient blood cell count depression. Fever, rigors and urticarial cutaneous eruption are common on the first or second day of ATG administration. These symptoms respond to antihistamines therapy and reduction of infusion speed. Anaphylaxis is rare but can be fatal. Corticosteroids 1 mg/kg are administered prior to each daily dose of ATG and continued after cessation of ATG therapy for four weeks slowly tapering the dose to prevent serum sickness. Steroids do not contribute to response in AA patients. Serum sickness typically occurs between days 7 and 14 from the start of ATG. Symptoms include rash, fever, myalgia, arthralgia, proteinuria and platelet consumption. The platelet counts should be maintained above 50.000/ μ L with transfusions during ATG administration because a rapid drop of platelets might appear under ATG infusion. The platelets should be administered before starting daily ATG dose. ATG is given for 5 days as a daily intravenous infusion over 12 to 18 hours through a central venous catheter. The doses and the duration of administration of different types of ATG are presented in the **Table 4. Different Types of Antithymocyte Globulin.**

The combination for the treatment of AA of an agent that lysis lymphocytes (ATG) with a drug that blocks lymphocyte function is rational and follows the observation that the more intense and prolonged the immunosuppression the higher success rate. CsA at a dose of 4-6mg/kg/day given for 12 months, in combination with ATG, was shown to be associated with a response rate of 71%. Slow tapering of CsA (0.3-0.7 mg/kg/month) resulted in significantly reduced incidence of relapse (8%) versus rapid (>0.8 mg/kg/month) tapering (60%). The CsA blood level should be maintained between 150 and 250 μ g/L, although there are no data on optimal levels.

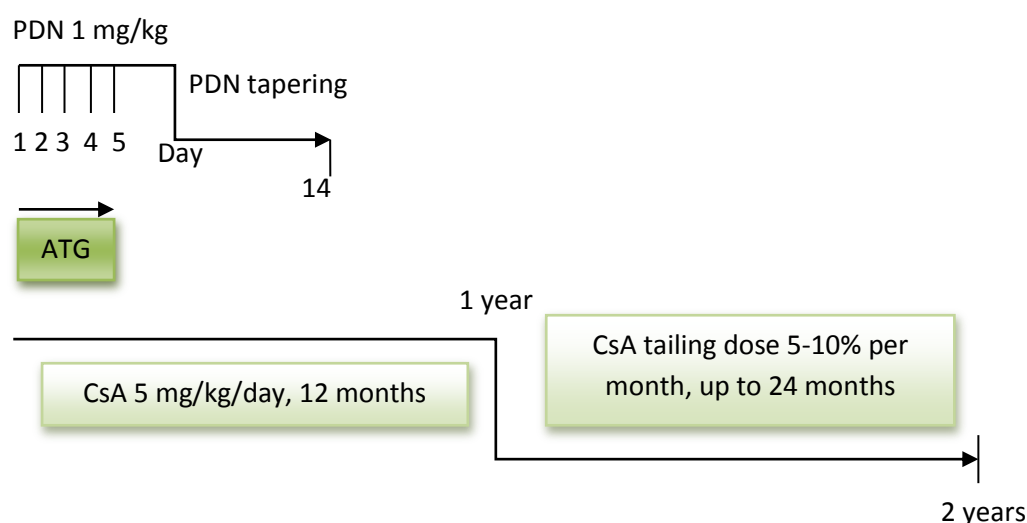


Figure 4. Combination immunosuppressive therapy protocol

Cyclosporine has considerable toxicity. Hypertension and renal toxicity are the most common serious side effects. Hirsutism and gingival hypertrophy are also frequent side effects. Increasing serum creatinine levels are an indication for dose reduction. The risk of nephropathy is increased by high doses and longer duration of therapy and occurs more often in older patients. Convulsions, possibly related to hypomagnesemia, are another serious complication of CsA therapy.

Infections are the principal cause of death during combination IST and having adverse effect on survival. In order to reduce infection related mortality, granulocytes stimulating growth factor (G-CSF) has been added to ATG and CsA. The current EBMT protocol recommends to use G-CSF only “on demand” during infectious episodes in neutropenic patients since there are some data reporting increased risk for clonal diseases after IST containing G-CSF.

In disease refractory to initial therapy with ATG and CsA, a repeat course as salvage regimen can produce a response rate of 30%.

Other immunosuppressants

Because half of patients with AA experience failure to IST, there have been many efforts to improve its efficacy by adding other agents. However, prospective studies conducted by the NIH group failed to show that the addition of mycophenolatemofetil or sirolimus to horse ATG/CsA results in improved overall response rate and reduced relapse rate.

Other immunosuppressants are used by some centers. High dose cyclophosphamide 45-50 mg/kg/day for 4 days without stem cell rescue is used by Johns Hopkins Group as treatment for patients with newly diagnosed AA with overall response rate claimed similar to that achieved with ATG + CsA. A prospective randomized study comparing this with ATG and CsA was terminated early because of excess deaths and fungal infections in the cyclophosphamide arm. Therefore, high dose cyclophosphamide without stem cell support is reserved for second or third line therapy. Mycophenolatemofetil has been used in organ transplantation and autoimmune disorders. There are data showing that addition of this drug to ATG and CsA did not improve response nor reduce the relapse rate. Alemtuzumab treatment has been retrospectively investigated in a limited cohort of 35 heterogenous patients with a response rate of 60%.

Immunosuppressive therapy in older patients

For older patients, consideration for treatment should be preceded by medical assessment to exclude significant co-morbidities and requires discussion of the risk with the patient. Although there is no upper age limit for ATG administration, CsA alone may be considered for patients older than 60 years. The current EBMT treatment algorithm proposes to treat hospitalized patients who are severely ill by ATG and CsA if considered to be tolerable considering the co-morbidity profile, whereas to start newly diagnosed patients who are well and in an outpatient setting with CsA alone.

The outcomes for tolerability and toxicity, response and relapse rates were examined in 24 older patients (over 60 years of age) receiving IST. Seven patients received standard IST consisting of standard-dose ATG with or without CsA, and 17 patients received attenuated IST consisting of at least a 50% dose reduction of ATG with CsA or CsA alone. Six patients (25%) had early deaths, mostly due to infection. Early mortality appeared higher in the standard IST group, although this was not statistically significant (43 vs 18%; $p = 0.4$). The 2-year cumulative incidence of response was 42% (95% CI: 26–69%). Responders had significantly better survival than non-responders ($p = 0.0002$). The 3-year probability of OS was 49% (95% CI: 27–68%). Nine out of 14 evaluable patients in the attenuated IST group had durable responses to treatment. These data from this small cohort suggest that attenuated dose IST could be a reasonable treatment option for patients deemed unfit for standard-dose IST.

Late Complication of Immunosuppressive Therapy

Relapse after IST is common. About 30% or more of the patients that responded require reinstitution of IST. Most relapse respond to retreatment, and there is no clear relationship with worse survival. Late complication of IST therapy with great impact on survival is the development of clonal hematologic disorders, especially myelodysplasia and acute myeloid leukemia. In the National Institutes of Health trials, the overall rate of clonal evolution is 12% to 15% at about one decade.

Over time, a minority of patients may develop a large clone of PNH and the haemolytic and thrombotic manifestations of PNH.

Immunosuppressive therapy for non-severe AA

There is only one prospective randomized trial of CsA alone or the combination of ATG/CsA treatments in patients with non-severe or moderate AA which showed that the outcomes of two groups of patients were comparable, with a 93% survival probability in the CsA group and 91% in the combination therapy group of patients.

Immunosuppressive therapy versus sibling matched donor BMT as frontline treatment

A recent analysis of outcomes from the EBMT comprising 2479 patients with SAA compared the survival after frontline treatment with IST versus BMT. The results showed a survival rate at 10 years of 73% in BMT recipients and 68% in those treated with IST ($p = 0.002$). It is important to mention that the frequency of secondary malignancy was much higher in the patients who received IST alone (1.2%) compared with those who received BMT (0.1%).

d. Supportive care in Aplastic Anemia

Supportive management for AA patients include all the measures necessary to prevent the occurrence of the redoubtable complications of pancytopenia like bleeding, infections and iron

overload. The overall survival has greatly improved for AA patients in the last 30 years due to prophylaxis and treatment of infections, bleeding prophylaxis, restrictive transfusion strategy, and iron chelation. The management of AA patients should be made by a multidisciplinary team and in a specialized center, especially the patients with severe neutropenia who are at risk for severe infections.

Bleeding

Platelet transfusions have greatly improved survival in patients with aplastic anemia and severe thrombocytopenia. Modern transfusion practice has made platelets safe to administer. The major problem related to platelet support is the development of alloimmunization in the recipient. Host antibodies directed against transfused platelet in the circulation shorten the transfused platelet life span and are almost always directed to HLA-A and HLA-B antigens. Alloimmunization is suggested by poor recovery of the 1-hour posttransfusion platelet count and confirmed by detection of specific HLA antibodies in serum. If HLA-antibodies are detected, HLA-matched platelets should be used for further transfusions. Alloimmunization can be prevented or delayed by the use of single-donor platelets rather than pooled platelets and by physical leukocyte depletion by filtration or ultraviolet treatment of blood products. Avoidance of platelet transfusions except when there is active bleeding is another alternative to prevent alloimmunization. The dose relationship between exposure to different donors' platelets and the probability of developing refractoriness is not clearly established. The risk for alloimmunization is thought to increase after more than 40 units of platelets have been administered.

Prophylactic transfusion of platelets is recommended in case of platelets $< 10 \times 10^9/L$ without fever, bleeding signs or history of major bleeding events. AA patients with fever, or bleeding signs or history of relevant bleeding like cerebral hemorrhage should receive prophylactic platelet transfusions in case of platelet counts $< 20 \times 10^9/L$. Bleeding prophylaxis must be individualized for each patient, but in general, a goal of maintaining platelet counts higher than $10 \times 10^9/L$ is reasonable. For invasive procedures platelet transfusions must be given to achieve the recommended levels. In case of patients receiving treatment with ATG the platelet counts should be increased to $50 \times 10^9/L$ prior to the onset of ATG infusions, as rapid drop of platelets might ensue under ATG infusion.

Prophylactic transfusions of platelets have not been shown to alter survival and their use should not be withheld if indicated just for fear of alloimmunization or increased risk of graft rejection after allogeneic stem cell transplantation. The beneficial effects of platelet transfusions meaning avoidance of bleeding complications and improvement of quality of life justify their use.

Prevention of bleeding complications may include some general measures like hormone therapy for women to control menorrhagia, avoidance of aspirin or other platelet aggregation inhibitor, and the use of tranexamic acid.

Anemia

The indication for erythrocyte concentrates (RBC) transfusions is hypoxic anemia. Symptomatic anemia develops at different haemoglobin levels depending on physical state and comorbidities of the patient. Therefore the decision to transfuse RBCs depends on clinical symptoms, haemoglobin level and quality of life. Frequent RBCs transfusions might result in the alloimmunization against erythrocyte antigens and iron overload. Iron chelation should be used in patients who are unresponsive to immunosuppressive therapy, necessitate transfusions over a long period of life and have a reasonable expectation of survival.

Alloimmunization secondary to transfusions increases the risk for graft rejection and mortality after BMT. Blood products from a potential bone marrow donor (sibling or parent) should be avoided. The transfusion of leukocyte-depleted blood products is mandatory in AA patients. The 5% risk of graft rejection after transplantation in untransfused patients was increased to 15 % with receipt of 1 to 40 units and to more than 25% in more heavily transfused patients. These data are from a period before leukocyte depleted blood products were routinely used and it is likely that graft rejection would be lower with the reduced patient alloimmunization by using leukocyte-depleted blood products. Further studies are needed to confirm this.

The irradiation of blood products for aplastic anemia patients is recommended during and after treatment with ATG or alemtuzumab as long as patients are immunosuppressed with a reduced CD4/CD8 ratio or a minimum of 6 months after IST and in case of patients receiving allogeneic BMT. The main reasons for using irradiate blood products are to prevent allosensitization and transfusion-associated GvHD. Also, granulocyte and HLA-matched platelets concentrates must be irradiated.

CMV-negative blood products are given in case of patients undergoing allogeneic SCT where both the patient and donor are CMV negative. In general, there is no need for CMV-negative blood products when leukodepletion is applied.

Granulocyte concentrates are used as a temporary measure and are reserved for the cases of life-threatening infections and severe neutropenia.

Infections

There is very little data regarding infections prophylaxis and treatment in AA. The recommendations are based on reports of infections in neutropenic patients with malignant diseases

and chemotherapy. The risk of infection is determined by the degree and duration of neutropenia. Susceptibility to infection is extremely high with an absolute neutrophil count (ANC) of less than 200 cells/ μ L. With longer periods of neutropenia, the probability of serious bacterial or fungal infections increases. Therefore, almost all severe AA patients unresponsive to IST are at high risk. Recommendations for initiation of empiric antibiotic therapy are similar in AA patients and other patients with neutropenia. A basic rule is, if ANC is less than 500 cells/ μ L and infection is suspected, immediate hospitalisation and broad-spectrum intravenous antibiotics should be institute. After the isolation of infectious agents in cultures or if new signs or symptoms of localized infection are identified the antibiotic regimen can be modified. Local hospital guidelines for treatment of febrile neutropenia should be followed. Usually, a combination of broad-spectrum beta lactam antibiotic and an aminoglycoside is the first choice. The patient's infection history and recent medication should also be taken into consideration. If the fever persists despite adequate antibiotic therapy or reappears, antifungal therapy should be given. Earlier addition of antifungals in AA patients is advisable if there are findings on chest tomography and a positive test result for galactomannan antigen. *Candida* and *Aspergillus* spp. are the most common agents incriminated for fungal infections in AA patients. The newer antifungal agents such as voriconazol or caspofungin, as studies have demonstrated, have the same or superior efficacy and less toxicity compared to amphotericin for the treatment of persistent fever in neutropenic patients and established *Candida* and *Aspergillus* infection. If a viral infection is suspected, an antiviral agent should be included in the treatment.

The use of G-CSF is recommended to be given for the treatment of infectious complications in AA patients. Because there are no guidelines and some studies have shown that prophylactic growth factors do not improve overall outcome, their use as infections prophylaxis is not advised.

Hospitalised patients with AA and severe neutropenia should ideally be cared for in isolation, in rooms with air filtration and should receive prophylactic antibiotics and antifungals.

Prevention of bacterial infections in severe neutropenic AA patients should be realised with a quinolone antibiotic or a combination of non-absorbable antibiotics such as neomycin and colistin depending on the local microbiological flora and rates of resistance.

Antifungal prophylaxis should be done in all patients with severe AA and prolonged neutropenia. Itraconazole, voriconazole or posaconazole appear to be more effective than fluconazole, as their activity against *Aspergillus* whereas fluconazole does not.

Antiviral prophylaxis and *Pneumocystis jiroveci* pneumonia (PJP) prophylaxis are not standardized. In case of allogeneic stem cell transplantation acyclovir and an anti –pneumocystis drug are routinely given. For patients treated with ATG the usage of acyclovir and PJP-prophylaxis is also a rule in many centers.

Iron chelation

It is now known that iron overload has a negative influence on the outcome of patients undergoing stem cell transplantation as well as a potential worsened effect on bone marrow function. Therefore serum ferritin level above 1000 µg/L requires starting iron chelation therapy. On the other hand due to potential side effects of chelate therapy a risk-benefit analysis should be assessed for individual patients. When remission is achieved an iron overload can be treated with phlebotomy.

Psychological support

The diagnosis of Aplastic Anemia has an important psychological impact on the patients, most of them being at a young age with life expectancies. It is a serious condition with potential fatal complications, a chronic disease and a life changing experience. The patient and its family must be informed about the nature of the disease, treatment, prognostic and social impact. Some patients may need professional psychological support.

Androgens

Testosterone and synthetic anabolic steroids were introduced in the 1960s and at that moment appeared to have major benefits in the treatment of AA. The high rates of response in early reports may be retrospectively attributed to the inclusion of patients with moderate acquired AA and constitutional AA. For severe AA, controlled trials have not demonstrated any benefits regarding survival rates or hematologic improvement. When added to immunosuppressive therapy androgens have failed to show an increase in response rates.

Androgens were preponderantly used and still are for second-line treatment of AA patients, but there had been a limitation of their use due to side effects such as hirsutism or hepatic toxicity and also due to restrictions of their availability and manufacture. Occasionally, there may be observed a hematological improvement with a course of androgen therapy, may be more effective if combined with CsA.

8. Prognosis

In the modern era, indicators for prognosis, besides the classic initial blood counts of a patient with AA, are considered to be absolute reticulocyte count and absolute lymphocytes predicting a good response to immunosuppression and survival. The sturdiness of the platelet and reticulocyte response after immunosuppressive therapy also correlates with long-term survival. Clonal evolution is a poor prognostic factor, especially the acquisition of monosomy 7. The rate of spontaneous recovery is low, untreated severe AA being invariably fatal.

Moderate AA, in contrast with the severe form, has a good prognosis. Some patients may experience a hematologic recovery with no treatment.

9. Conclusions

The outcome of patients diagnosed with acquired Aplastic anemia has greatly improved over time. Age remains a major predictor of outcome for both BMT and immunosuppressive treatment. Early intervention is associated with a significantly superior outcome. Improved survival is seen both in patients grafted from a sibling matched donor, and those with a unrelated matched and alternative donor, suggesting that better supportive care and improved management of infections have an major impact on outcome.

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