Diagnosis and Treatment of Acquired Aplastic Anemia

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KEYWORDS

• Antithymocyte globulin • Hemopoietic stem cell transplantation
• Bone marrow failure • Cyclosporine • Growth factors

The clinical symptoms of acquired aplastic anemia (AA) depend on the degree of cytopenia and on the time required for it to develop. Severe cytopenia may cause fever, bleeding, and life-threatening infections: often these are young patients, otherwise healthy, sometimes with a preceding history of a recent febrile illness, and occasionally following an episode of elevated serum transaminases with or without cholestasis. These patients require immediate high-intensity care, with appropriate transfusions and antimicrobial therapy and HLA-typing if under the age of 40. An episode of sepsis or a cerebral hemorrhage can be fatal in the first weeks of presentation for these acute patients.

When the cytopenia is less severe, patients are in better clinical condition, and they may also be asymptomatic. Some of these patients are seen by their general practitioner for mild cytopenia, and eventually demonstrate declining peripheral blood counts with time, often over a period of many months. At that point, and especially when their anemia requires red blood cell (RBC) support, they may be referred to the hematologist, who starts a thorough work-up.

DIAGNOSIS

The diagnostic criteria of acquired AA have been defined, and require an empty or hypoplastic marrow, with peripheral blood cytopenia. Severe AA (SAA) is present when patients meet at least two of the following criteria: and absolute neutrophil count less than 0.5 $\times$ 10^9/L, platelet count less than 20 $\times$ 10^9/L, or reticulocytes less than 20 $\times$ 10^9/L.

The diagnosis of acquired AA also requires the exclusion of other conditions associated with pancytopenia, however, among these congenital marrow failure syndromes, such as Fanconi anemia, and myelodysplastic syndromes (MDS) or

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leukemia. Fanconi anemia can be suspected by examining the patient, especially if a child or a teenager, and can be excluded by a chromosomal breakage test using either peripheral blood lymphocytes or dermal fibroblasts exposed to di-epoxibutane or mitomycin C.[1]

Fanconi anemia cells show excessive stress-induced chromosomal breakage. This test is not only of value in children with bone marrow failure, but also young adults can be found to have Fanconi anemia. Some rarer congenital marrow failures, without specific markers, can be more difficult to exclude. These disorders are discussed in detail elsewhere in this issue. MDS can be ruled out by appropriate marrow cytology-histology and cytogenetic analysis, and the same is true for acute leukemia. The distinction between Fanconi anemia, MDS, and AA is important, because treatment is different in these three conditions. Clonal cytogenetic anomalies alone, if the presentation is otherwise typical of AA it is not sufficient to diagnose hypoplastic MDS. Such cases should be treated along the guidelines established for AA (discussed elsewhere in this issue). In the presence of an empty marrow, pancytopenia, and transfusion dependence, the severity of the disease is based on neutrophil (polymorphonuclear [PMN]) count: nonsevere AA (PMN >0.5 $\times$ 10^9/L); SAA (PMN 0.2–0.5 $\times$ 10^9/L); very SAA (vSAA) (PMN <0.2 $\times$ 10^9/L). As will be seen, this may no longer be the case with current treatment strategies.

PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

Paroxysmal nocturnal hemoglobinuria (PNH) in its classic hemolytic form is quite different from AA: patients have a full marrow, with erythroblastic hyperplasia, and white blood cell and platelet counts are normal. In addition, patients complain of episodes of nocturnal hemoglobinuria with dark gray urine in the morning and their ferritin levels are low, because of urinary losses. The expression of glycosyl-phosphatidylinositol on the surface of granulocytes, monocytes, lymphocytes, and erythrocytes is deficient or lacking in classic PNH patients. Some AA patients may have a deficient expression of glycosyl-phosphatidylinositol–liked antigens, however, without hemolysis and without hemoglobinuria: these patients are referred to as ''AA-PNH.'' It is important to recognize these two different clinical settings, because classic PNH and AA-PNH are treated differently: the former may be eligible for eculizumab therapy[2] or allogeneic stem cell transplantation.[3] The latter is treated like any other patients with acquired AA. Several reports have suggested that the presence of a so called ''PNH-clone'' (a small percentage of cells with defective glycosyl-phosphatidylinositol–associated antigens) is a favorable prognostic predictor for response to immunosuppressive therapy (IS).[4] PNH is reviewed in more detail elsewhere in this issue.

TREATMENT OF ACQUIRED APLASTIC ANEMIA

Supportive Care

Before discussing definitive treatment of acquired AA, one should first consider supportive care. This depends on the severity of cytopenia, and may include RBC transfusions, platelet transfusions, antibiotics, antifungal agents, and antiviral therapy. RBC transfusions should be filtered to decrease the likelihood of alloimmunization and reduce the incidence of viral infection. Whether irradiation of blood products is necessary is not known; many centers do provide irradiated blood products to these patients while undergoing IS treatment and stem cell transplantation. Although it is unlikely that RBC transfusion causes graft-versus-host disease, nevertheless irradiated blood products have been shown to reduce alloimmunization in prospectively

transfused animals.[5] Candidates for an allogeneic transplant may benefit from a program of irradiated RBC and platelet transfusions. Antimicrobial therapy depends on the severity of neutropenia and on the presence of symptoms and signs of infection: one should, however, consider the fact that AA patients may present after a history of neutropenia or may face a long period of neutropenia. In these conditions fungal infections, and especially invasive aspergillosis, are known to correlate with the duration of neutropenia, and should warrant the initiation of an early therapy with mold-active drugs. Appropriate transfusion support and antimicrobial treatment may be essential in the early phases of diagnosis and treatment planning. Most centers also provide anti–herpes virus prophylaxis. The use of growth factors is equally controversial; whereas granulocyte colony–stimulating factor (G-CSF) may be of use in neutropenic infections the uncritical use of growth factors may also delay the initiation of appropriate therapy.

Designing a Treatment Strategy

As a general rule treatment is indicated when peripheral blood counts drop to levels that call for RBC or platelets transfusions. Patients with moderate cytopenia not requiring transfusions, also referred to as "hypoplastic anemia," should be followed closely to ascertain whether there is deterioration with time. During this first period they can be offered supportive care or outpatient treatment with anabolic steroids or low-dose steroids or cyclosporin (CsA). Recently, androgens have been shown to increase telomerase activity in human $CD34^+$ cells and this may provide an explanation for their effect, sometimes striking, in patients with AA.[6] This is in keeping with a trial showing increased responses in the group of patients randomized to receive androgens together with IS therapy.[7]

Patients with cytopenia requiring transfusions should be treated as inpatients, with either IS therapy or bone marrow transplantation (BMT), and decision to start treatment should not be delayed, because this may significantly impact on the chance of success.[8] The choice between these two latter treatments is based on severity of the disease and patient age: young patients (<20 years) with vSAA are candidates for first-line transplantation. Older patients with moderate disease are generally offered IS therapy as initial therapy.[9]

IMMUNOSUPPRESSIVE TREATMENT

Combination Therapy

Treatment with antithymocyte globulin (ATG) yields superior survival when compared with supportive care.[6,10] Combinations of ATG with androgens[7] or CsA[11] improves the overall response rates but does not improve survival. The update of the German study comparing ATG + CsA with ATG alone shows a difference in event-free survival, indicating that patients receiving ATG alone required additional courses of IS, as compared with patients receiving ATG + CsA; however, survival at 15 years was comparable.[12] Event-free survival is a relevant outcome, because it indicates survival without transfusions and without additional courses of ATG, and this is relevant for quality of life.[12] The median time to achieve a response is 120 days,[13] and a second treatment should not be planned earlier than 4 months after the initial ATG treatment. Responses can be subdivided into complete and partial: the former requires normal blood counts, although some reports indicate complete response as patients with a hemoglobin greater than 10 G/dL, a PMN count greater than $2 \times 10\text{^}9/L$, and platelets greater than $100 \times 10\text{^}9/L$.[13] Partial responses require at least transfusion

independence. The probability of becoming transfusion independent varies from 40% to 80% according to the IS regimen and the patient population.

Horse Versus Rabbit Antithymocyte Globulin

Both horse ATG and rabbit ATG have been used successfully in patients with acquired AA.[6] The standard first-line IS treatment is currently horse ATG + CsA and second-line treatment is rabbit ATG + CsA, although the latter has been successfully used also as first-line therapy.[14,15] The infusion of ATG may cause allergic reactions, but with appropriate premedication with steroids or antihistamines, and appropriately slow infusion (up to 24 hours for each dose), almost all patients can complete the prescribed total course of ATG, usually lasting 5 days. ATG does have a first dose effect probably related to cytokine release by lymphocytes and it is a common error to stop treatment for ''intolerable'' fever and rigors. Infections, hemorrhages, and fever are not an absolute contraindication for treatment with ATG: although these antibodies make cytopenia worse in the first weeks, they should be considered as necessary therapy, just as chemotherapy is required for leukemic patients presenting with cytopenia. In a recent analysis, patients treated beyond the median interval between diagnosis and IS of 23 days have significantly greater risk of failing in multivariate analysis.[8]

Cyclosporin Dependence and Relapse

Current IS regimens including CsA call for full CsA dose (5 mg/kg/d orally) for 6 months; after this time point CsA is tapered, and it is unclear exactly when and how fast this should be done. A recent study of the Italian pediatric group has addressed these two questions. Forty-two children were divided into three groups: (1) very slow tapering (<0.3 mg/kg orally); (2) slow CsA tapering (0.4–0.7 mg/kg orally); and (3) rapid tapering ($\geq$0.8 mg/kg orally). The cumulative incidence of relapse was 8% in the slow–very slow taper group and 60% in the rapid-taper group.[16] Among patients who eventually discontinued CsA, the median duration of CsA treatment at full therapeutic dose (4–6 mg/kg) was 12 months (range, 3–45) and tapering was completed in a median of 19 months (range, 4–64 months). In that study the actuarial probability of discontinuing CsA was 21% at 5 years, 38% at 7 years, and 60% at 10 years, respectively.[17] This study suggests that it is safe to start tapering CsA at 12 months of treatment (rather than 6), and that tapering should be very slow (less than 10% of the dose per month) for at least 1 year to minimize the risk of relapse. Other groups do, however, taper CsA more rapidly and the issue has to be considered as open. Furthermore, little data are available on appropriate drug levels in this disease.

Relapse

Relapse is defined as a patient requiring transfusions of RBCs or platelets after having been independent from transfusion for at least 3 months.[17] The risk of relapse in the pre-CsA era was 35% and was not easily predicted;[17] in a recent Japanese prospective study the risk of relapse was significantly higher in patients receiving ATG + CsA (42%) compared with patients receiving ATG + CsA and G-CSF (15%) (P = .01),[18] although 4-year survival was not significantly different (88% versus 94%).[18] This indicates that response can be achieved after hematologic relapse, with an additional course of ATG.[17,19]

Growth Factors

The use of G-CSF has been described in conjunction with ATG and CsA as first-line treatment.[14] The potential advantages of using G-CSF are faster neutrophil recovery,[20] and the opportunity to test for white blood cell increments and to predict

early responses and early failures.[14] The authors have recently confirmed that achieving a white blood cell count of 5 × 10^9/L while receiving G-CSF in the first 3 months is predictive of response and survival. Patients who do not achieve a white blood cell count of 5 × 10^9/L have a 72% chance of not responding, a 79% probability of failing primary therapy, and an 84% risk of death.[21] The use of G-CSF allows early identification of nonresponders and early referral for BMT.

The use of daily G-CSF for 4 months, however, has drawbacks. Such treatment is costly and survival was not improved in G-CSF patients in two prospective randomized trials.[18,20] Interestingly, both randomized trials showed no difference in the risk of late clonal disorders in patients receiving or not receiving G-CSF.[18,20] This is in contrast to a retrospective European study showing a borderline increased risk for G-CSF patients,[21] and the Japanese retrospective study also showing increased risk of clonal disorders.[22] A correlation was also found between duration of exposure to G-CSF and clonal disease.[22] The cause-effect relationship is unclear, however, because nonresponders to IS are more likely to develop clonal disorders,[14] and prolonged G-CSF is given precisely to these patients. A third randomized study has been completed by the European Group for Blood and Marrow Transplantation (EBMT) and will be analyzed in the next months. Finally, a further increase of G-CSF to 10 μg/kg/d failed to show any advantage over the conventional 5 μg/kg/d.[23]

Clonal Evolution and Second Malignancies

There are several possible evolutions of the disease that may or may not have to do with its etiology, pathogenesis, and treatment. The appearance or the increase of glycosyl-phosphatidylinositol–negative subpopulations or the evolution toward frank PNH, with increasing levels of L-lactate dehydrogenase, and increasing spleen volume, may be confirming the link between the two disorders.[3] The appearance of cytogenetic clonal abnormalities (eg, trisomy 8) may suggest a pre-existence clone at diagnosis, or the emergence of a +8 clone during stressed hematopoiesis. Some AA cases may proceed to develop frank acute leukemia. The overall risk of developing a clonal cytogenetic abnormality and MDS at 10 years is set between 5% and 20%, and may well depend on the degree of response to IS.[14] An important contribution to this problem was a study by the EBMT published in 1993,[24] comparing second malignancies in patients with AA treated with either IS or BMT. The risk of MDS and acute myelogenous leukemia was significantly higher for IS as compared with BMT patients, suggesting that MDS and acute myelogenous leukemia follows IS treatment, rather than being present at diagnosis: this is because cyclophosphamide (CY), 200 mg/kg, used in transplantation, is unlikely to eradicate a neoplastic clone. Second tumors (especially of the mouth) were frequent in patients receiving radiation before BMT. This is why currently HLA-identical sibling transplants should not receive standard dose radiation. Very low dose (2 Gy) total body radiation (TBI) is being explored in patients undergoing an alternative donor transplant.[25] The factors that may play a pathophysiologic role in clonal evolution in patients with marrow failure states are reviewed elsewhere in this issue.

Factors Predicting Response to Immunosuppressive Therapy

The importance of predicting response is relevant for every disease, but in AA patients, pancytopenia has acute consequences, with daily risk of blood-borne infections or cerebral hemorrhages, and this makes predictive factors very important. In a recent study on almost 1000 patients treated in Europe between 1991 and 2002, the strongest negative predictor was age (>16 years) (RR 1.76, $P = .0009$), followed by an IS protocol other than ATG + CsA (RR 1.29, $P = .02$) and interval between diagnosis

and treatment over 23 days (RR 1.32, $P = .04$).[8] The year of IS treatment (before or after 1997) had a borderline negative effect,[8] suggesting little overall improvement in this last 5 years. Of interest is the fact that severity of the disease, as identified by PMN counts (<0.2, 0.2–0.5, >0.5 × 10^9/L), had no impact on survival, in contrast with results from the original 1988 analysis of the EBMT, showing that PMN count was the strongest predictor of survival.[26] Results have improved dramatically especially in children with vSAA, from 37% in the 1980s to 83% in the 1990s,[8] but this has not been the case for SAA and actually the non-SAA patients showed a trend for worse outcome.[8] Currently, survival can be predicted by age: the 10-year actuarial survival is 73% for patients less than 20 years, 75% for patients aged 21 to 30, 66% for patients aged 31 to 40, and 47% for patients aged over 40 years.

Immunosuppressive Therapy for Older Patients

The author have analyzed the outcome of older patients treated with ATG and cyclosporin:[27] the actuarial 10-year survival was 80% for patients aged 0 to 20 and 21 to 50, it was 45% for patients aged 51 to 70, and it was 25% for patients over the age of 70.[27] Nevertheless, the standardized mortality ratio, indicating the ratio between mortality of patients and of an age-matched population, is 33, 14, and 9, respectively, for the age group less than 50, 50 to 70, and greater than 70.[27] These data suggest that the corrected risk of death is highest in young patients and gets progressively lower with increasing age.

Pregnancies After Immunosuppressive Therapy

Counseling a young woman in remission following IS about the risks of pregnancy is a difficult task. There is only one study of relevance from the EBMT, and it demonstrates that successful pregnancy is possible, but the risk of relapse is approximately 20%. In that study, one of the relapses was fatal.[28] Relapses were more frequent in partial responders as compared with complete responders. If a young woman is in complete remission, off therapy, and is informed about the risk of relapse of the disease, and if she wishes to become pregnant, she can be counseled that a pregnancy is possible.

BONE MARROW TRANSPLANTATION

It is well known that young patients grafted from an HLA-identical sibling have the best outcome, when compared with older patients allografted from alternative donors. A recent study on 1567 patients has confirmed these results: significant predictors of survival were year of transplant greater than 1997, matched sibling donor, age less than 16 years, interval between diagnosis and transplant of less than 83 days, and a conditioning regimen without radiation.[8]

HLA-Identical Siblings

The current survival of children (<16 years) receiving a BMT from an HLA-identical sibling is 91%, and it is 74% for patients older than 16 years.[8] This is true for patients receiving CY, 200 mg/kg, and a BMT. A recent EBMT/IBMTR report suggests that the use of peripheral blood as a stem cell source reduces survival when compared with bone marrow in patients less than 20 years from 85% to 73%, and in patients greater than 20 years from 64% to 52%.[29] The major cause of excess mortality in the peripheral blood arm was chronic graft-versus-host disease. This study would not recommend peripheral blood as a stem cell source in patients with acquired AA, possibly because an increased incidence of chronic graft-versus-host disease does not benefit

aplastic patients in the way it might benefit patients with leukemia. A suitable marrow cell dose is recommended, because results of transplantation are highly dependent on the number of nucleated cells infused.[30] CY alone remains the best conditioning regimen for young patients, and ATG seems to reduce the risk of graft failure,[31] although a recent randomized trial has shown some advantage, but no significant difference in survival.[32] The combination of CsA and methotrexate seems to offer a survival advantage over CsA alone (84% versus 75%) used for graft-versus-host disease prophylaxis.[33] Standard CY, 50 mg/kg/d $\times$ 4, with 3 days of ATG conditioning, followed by unmanipulated marrow as a stem cell source and CsA and methotrexate as graft-versus-host disease prophylaxis is still standard of care for young patients with acquired AA undergoing an HLA-identical sibling transplant. The use of radiation, peripheral blood, or other conditioning regimens should all be tested in prospective trials, because of their unproved benefit for the patient.

HLA-Identical Bone Marrow Transplantation for Patients Over 30 Years

Notwithstanding the excellent results with a standard BMT in AA, there is still a strong age effect: the actuarial 10-year survival for patients grafted from HLA-identical siblings in the last decade is, respectively, 83%, 73%, 68%, and 51% for ages 1 to 20 (N = 681), 21 to 30 (N = 339), 31 to 40 (N = 146), and 40 and older (N = 111) (R. Oneto, unpublished EBMT data, 2008). Unfortunately, the use of peripheral blood as a stem cell source not only did not solve the problem, but outcome was actually worsened.[29] The authors have explored the use of low-dose CY (30 mg/m^2 $\times$ 4) in combination with low-dose fludarabine (FLU) (30 mg/m^2 $\times$ 4) and ATG in patients over the age of 30. The initial results are encouraging, with transplant mortality occurring in 30%, but more patients need to be accrued (S. Maury, unpublished EBMT data, 2008). There have been three reports in the literature confirming that the combination FLU-CY produces a high rate of engraftment and very encouraging survival.[34–36] The median age of these patients was actually younger than 30, and the dose of CY higher (60–120 mg/kg), but this new regimen, which derives from the original experience of the Houston group,[37] seems very encouraging.

Unrelated Donor Transplants

The outcome of unrelated donor transplants for patients with AA has improved in the last decade.[38,39] Better selection of HLA matched donors has probably played a major role, but also significant changes in the conditioning regimen have occurred.[25,40,41] The first of these studies tested de-escalating doses of radiation, from 6 Gy down to 2 Gy, and concluded for best results for patients receiving 2 Gy, with 8 of 13 patients surviving.[40] The Japanese study reported 154 SAA patients undergoing an unrelated donor transplant, most receiving 3 Gy TBI:[25] unfavorable factors for survival were older age (>20); conditioning without ATG; and a long (>3 years) interval from diagnosis to transplant. The EBMT study tested a nonradiation-based program:[41] results were overall encouraging with 70% surviving, although rejection was high in young adults over the age of 14. The EBMT is currently testing a conditioning regimen that is very similar to the Japanese regimen: FLU-CY-ATG and low-dose TBI (2 Gy) (A. Bacigalupo, unpublished EBMT data, 2008).

As a consequence of less toxic conditioning regimens and improved donor-recipient matching, survival has almost doubled in the past decade[8,38] from 38% in 1991 to 1996 to 65% in the period 1997 to 2002,[8] and in the latter period survival after unrelated donor transplants in children is 75% versus 63% for adults greater than 16 years of age.[38] Results of unrelated donor transplants have improved to such an extent that treatment strategy may be affected: in children without a matched sibling donor, an

unrelated donor search should be started at diagnosis, and transplantation should be seriously considered after one course of IS in the presence of a suitable donor. In young adults between 20 and 30 the same may be true. Adults over the age of 30 should be entered on a prospective trial. Alternative donor transplant is an option for second-line treatment in patients failing one or two courses of IS treatment.

Cord Blood Transplants

A proportion of patients does not have a matched donor in the family, and cannot find a suitable unrelated donor in the world wide network (bone marrow donors world wide). The percentage of these patients who lack a donor varies between 5% and 40%, according to the ethnic origin of the patient. Cord blood transplants is an alternative option that has been successfully explored in patients with hematologic malignancies.[42] Because of the high rate of rejection in AA patients and the low cell numbers of cord blood units, transplants of unrelated cord blood has usually been discouraged in this setting. A recent study from the Japanese group,[43] however, reports 31 cord blood transplants with an overall survival of 42%, but a more encouraging 80% survival for patients receiving the FLU-CY-TBI 2-Gy combination as a conditioning regimen. Cord blood may not be the first option in AA patients lacking a family donor, but some investigators are exploring this stem cell source, and results may be encouraging with appropriate cell dosing, double units, alternative routes of administration, and new conditioning regimens.

FIRST-LINE THERAPY

Table 1 summarizes first- and second-line therapy. First-line therapy is identical in patients up to the age of 40: BMT from identical sibling if available, ATG + CsA if

Table 1
Summary of first- and second-line treatment in patients with severe or very severe acquired aplastic anemia

	First-Line Treatment	Second-Line Treatment	Other
<20 y, sibling donor	Sibling BMT	—	—
<20 y, no sibling donor	ATG + CsA MUD search	MUD transplant CB transplant Second ATG + CsA	—
20–40 y, sibling donor	Sibling BMT	—	—
20–40 y, no sibling donor	ATG + CsA MUD search	Second course ATG + CsA MUD transplant	Third course ATG MUD transplant
>40 y, sibling donors	ATG + CsA	Sibling BMT	—
>40 y, no sibling donor	ATG + CsA	Second course ATG + CsA MUD search	MUD transplant Third course ATG
Relapse after response to ATG + CsA	ATG + CsA	MUD transplant	—
No response to two courses of ATG + CsA, and no donor	—	—	Supportive care Experimental IS BMT from an alternative donor

Abbreviations: ATG + CsA, Antithymocyte globulin and cyclosporine; BMT, Bone marrow transplantation; CB, Cord blood; IS, Immunosuppression; MUD, Matched unrelated donor.

Box 1
Management of acquired aplastic anemia

1. Establish correct diagnosis
2. Initiate early supportive care
3. Perform HLA typing of patient and family members early
4. Activate search for an unrelated donor, when a family donor is not available
5. Treat early with either BMT or IS
6. Do not stop ATG treatment simply because of allergic reactions
7. Enter patients in innovative prospective trials

an identical sibling not available. Second-line therapy, however, differs: young patients (<20 years) may proceed to matched unrelated donor BMT, whereas older patients (>20 years) should probably receive a second course of IS, and continue the search for an unrelated donor. An alternative donor transplant can be considered, however, also in patients over the age of 40, if there is heavy transfusion requirement and one believes that further IS may only be delaying a transplant. Over the age of 40 a sibling transplant is usually second line, and matched unrelated donor transplant third line. Patients relapsing after remission from ATG + CsA should receive one course of IS therapy. Patients refractory to two courses of ATG may be entered in prospective protocols testing new agents or experimental transplant procedures.

SUMMARY

Acquired SAA can be treated successfully with either IS therapy or BMT (**Box 1**). Although IS can be readily administered to all patients, it is not a curative approach. In contrast, BMT produces rapid and long-lasting hematologic recovery, without the long-term risk of MDS, but requires a suitable donor and carries the risk of transplant-related mortality.

Survival after BMT from HLA-identical siblings in young patients exceeds 90%, after a conventional CY conditioning, and unmanipulated bone marrow, which is still preferred over peripheral blood as a stem cell source. In some countries patients come to stem cell transplant with a heavy transfusion burden, and highly sensitized. The conditioning regimen may need to be modified in this situation, with the addition of low-dose TBI or low-dose busulfan. Similarly, BMT in patients over the age of 30 may require changes in the conditioning regimen, such as the combination of FLU-CY-ATG. This combination together with low-dose TBI has become the standard regimen for alternative donor transplants in Europe and Japan and is being tested in the United States. The excellent result calls for an unrelated donor search, early in the course of the disease. Age remains a major predictor and requires careful consideration when deciding the treatment strategy.

If one chooses to start with IS therapy, then ATG + CsA remains the preferred first-line combination. For IS patients, age and interval from diagnosis are two major predictors, whereas a low neutrophil count is no longer a negative indicator. This is because of the improved outcome in vSAA patients treated with combined ATG + CsA, but not in patients with less severe AA. The advantage of giving G-CSF, together with ATG + CsA, has been disputed by two randomized trials. In patients responding to IS, CsA should be tapered very slowly over a period of 1 year. Finally, a short interval

between diagnosis and treatment is associated with better results so patients should be referred promptly to experienced centers. In addition, AA is a rare disease and patients should participate in well-designed prospective clinical trials.

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