GUIDELINES FOR THE TREATMENT OF SEVERE APLASTIC ANEMIA

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This paper provides an algorithmic approach to the management of patients with severe aplastic anemia (SAA). Although SAA remains a serious hematological disorder, clinical results have been steadily improving over the past two decades, and we now have clear indications as to optimal treatment modalitites. The present guidelines are outlined to improve diagnostic accuracy and to help the physician choose the best treatment for an individual patient with SAA. We have selected data from the SAA Working Party (WPSAA) Registry, from the literature and from our own experience.

These data reflect a consensus among members of the WPSAA and of the *European Group for Blood and Marrow Transplantation* (EBMT). The WPSAA keeps a data base of patients with SAA treated with bone marrow transplantation (BMT) or immunosuppression (IS); the Registry now contains over 2000 patients and can be used for retrospective analyses.

Due to the rarity of this disease and the number of unresolved problems it continues to present, it is also important that patients be treated within one of the ongoing clinical trials.

Definition

The term *acquired severe aplastic anemia* (SAA) applies to patients with anemia requiring red blood cell support, and/or with thrombocytopenia requiring platelet transfusions, along with one of the following:[1] a hypoplastic marrow without blasts; a neutrophil count of $<0.5\times10^9$/L; a platelet count of $<20\times10^9$/L. A subgroup of patients merits the category of very severe AA when the PMN count is $<0.2\times10^9$/L.

For practical purposes and for inclusion in ongoing international protocols, we subdivide patients according to their PMN counts into two major risk categories: PMN $\leq0.5\times10^9$/L and $>0.5\times10^9$/L.

The term *non-severe aplastic anemia* should be reserved for patients with moderate cytopenia not requiring blood cell support; these patients will not be discussed in the present report.

Diagnostic algorithm for SAA

The patient usually presents to the general practitioner or to the hematology ward with pancytopenia. We have defined the level of pancytopenia required for a diagnosis of SAA, and we feel the following additional investigations are mandatory in a diagnostic approach (Figure 1).

Etiology

First clinical examination, presentation of the disease and past clinical history are helpful in exploring the possible etiology of the disease: it is not uncommon to have a previous history of hepatitis, though association with the hepatitis virus has been disputed.[2] Some drugs have also been associated with SAA.[3] Most of the patients, however, will be classified as having idiopathic, or unknown etiology SAA.

Morphology

Bone marrow morphology is the first prerequisite for an initial correct approach to patients with cytopenia: a bone marrow biopsy with good histology is absolutely essential for excluding acute leukemia or a myelodysplastic syndrome (MDS). Bone marrow and peripheral blood cytology complete the morphologic approach.
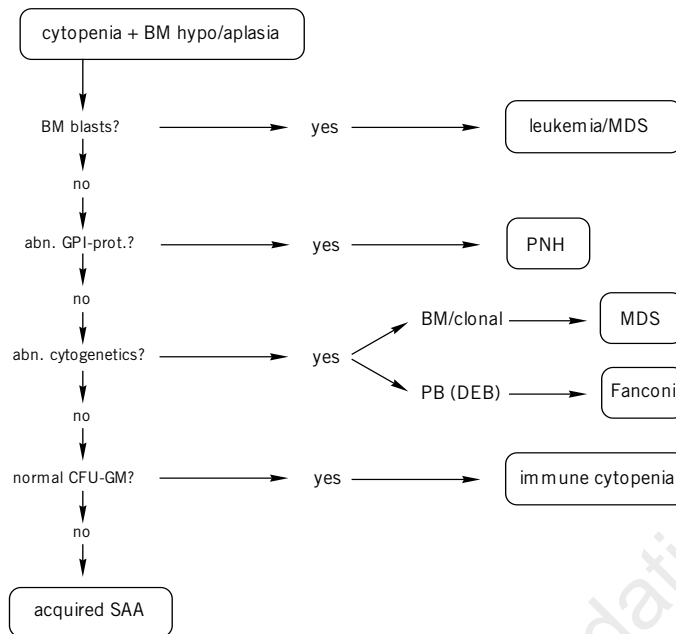
Figure 1. Diagnostic approach to the cytopenic patient: this includes marrow histology for cellularity and blast cells, FACS analysis of glycosyl phosphatidyl-inositol (GPI)-linked proteins (CD14, CD16, CD58, CD59), cytogenetics of marrow cells and peripheral blood lymphocytes after exposure to DEB and, finally, in vitro growth of hematopoietic progenitors. Abbreviations: BM=bone marrow; MDS= myelodysplasia; PNH= paroxysmal nocturnal hemoglobinuria; PB= peripheral blood; DEB=diepoxybutane; CFU-GM= granulocyte-macrophage colony forming units.

GPI-linked proteins

The expression of glycosyl phosphatidyl-inositol (GPI)-linked proteins should be normal. These include CD14 on monocytes, CD16 on granulocytes, CD58 on erythrocytes and CD59 on lymphocytes. The lack of expression of GPI-linked proteins in one or more cell lines should raise the possibility of paroxysmal nocturnal hemoglobinuria (PNH). This is usually, but not consistently, associated with signs of hemolysis, with hemoglobinuria and with a positive Ham test.[4]

Cytogenetics

Cytogenetic analysis of bone marrow cells should reveal a normal karyotype.

A clonal abnormality, such as trisomy 8 or loss of chromosome 5 or 7, would suggest MDS. In young children a karyotype from PHA-stimulated peripheral blood lymphocytes, after exposure to diepoxybutane (DEB), should also be performed to exclude Fanconi's anemia.

In vitro colony formation

Finally, colony formation of hemopoietic progenitors from the marrow should be very low or absent; if this is not the case, one should consider an immune mediated cytopenia.

Therapeutic options for SAA

There are two basic therapeutic approaches in SAA: transplantation or pharmacological treatment without BMT.

Bone marrow transplantation

Syngeneic transplants. Syngeneic bone marrow transplants for SAA are not frequent; nevertheless, they have highlighted some of the pathogenetic problems in this disease. Indeed if SAA were caused by a quantitative defect of stem cells as occurs in accidental exposure to ionizing radiation, then the simple infusion of normal syngeneic stem cells should lead to hemopoietic reconstitution. This is not always the case; and in fact there are very few patients

in the EBMT data base for whom infusion of syngeneic marrow has led to permanent hematologic recovery. Most patients require high-dose chemotherapy prior to transplantation in order to allow engraftment.

This suggests that most patients with SAA also have a problem within the accessory cells, possibly within their immune system.[3,5-8] A very particular patient has been described by J. Hows (personal communication).

This was an elderly female with SAA who received syngeneic marrow after cyclophosphamide preparation. She engrafted and rejected the marrow; she was then prepared again with cyclophosphamide and total body irradiation, engrafted and rejected again. A third infusion of syngeneic marrow was attempted after further conditioning, but the patient died of infection. This extreme case is an example of how the immune system of a person with SAA is capable of rejecting normal syngeneic stem cells, suggesting that the immune system is heavily involved in the pathogenesis of the disease.[3]

Eighteen syngeneic transplants conditioned with cyclophosphamide (200 mg/kg) have been performed in Europe and all these patients, with the exception of the elderly woman just described, are still surviving with a median follow-up of 5 years (unpublished). Syngeneic transplantation is indicated up to the age of 50 years.

HLA-identical sibling transplants. Significant progress has been made in HLA-identical sibling transplants over the past two decades: survival has improved from 37% in 1970-79 to 62% in 1980-83 to 68% in 1984-93. Improvement has been obtained by reducing graft rejection, thus decreasing failures due to infections. The rejection rate has been 24%, 8%, 7%, respectively, in the three transplant eras described.[9]

The major reason for this improvement has been the use of cyclosporin A (CyA). In multivariate analyses patients receiving cyclophosphamide for conditioning and CyA for graft versus host disease (GvHD) prophylaxis had a significant survival advantage (EBMT analysis, 1991). The use of irradiation also reduces the incidence of graft rejection, but is associated with increased early toxicity like

GvHD and interstitial pneumonitis (IP), and late sequelae such as chronic GvHD and secondary tumors.[10]

Cyclophosphamide for conditioning and CyA for GvHD prophylaxis is currently the best protocol for HLA-identical sibling transplant. This is true for patients who are unsensitized at transplant, as identified by appropriate platelet increments after random platelet transfusions. For patients have been heavily transfused and therefore are highly sensitized, it may be appropriate to increase pre-transplant immunosuppression by adding antilymphocyte globuline (ALG) to cyclophosphamide,[11] although the short-term and possibly long-term consequences of increased immunosuppression have not yet been fully evaluated. An other important variable is patient age: 5-year actuarial survival is currently 70%, 62% and 30%, respectively, for patients aged 0-19, 21-40 and over 40 (unpublished EBMT data). In pediatric age survival appears to be superior in very small children (0-5 years: 95% survival) as compared to older children (6-10: 72%; 11-15 years: 62%) (EBMT data unpublished).

Transplants from non HLA-identical donors. Graft failure has been a significant problem in transplants from HLA-identical siblings and still is for approximately 10% of patients. This is due to the fact that most recipients are prepared with cyclophosphamide alone, and also perhaps because of the intrinsic nature of the disease. Intensification of the conditioning with irradiation has significant short-term and long-term consequences. No wonder therefore that graft failure is a major problem in transplants from non HLA-identical donors. A number of such transplants have been reported: the EBMT-WPSAA shows acceptable long-term results only in the presence of six-antigen-matched donors.[12] Overall results are clearly inferior to immunosuppression (IS) or HLA-identical BMT. Some groups have used very intensive conditioning regimens with some encouraging results.[13] Some anecdotal successful transplants across the HLA barrier have been reported in children.[14] The *International Matched Unrelated Study* (IMUST) recently presented data suggesting improved results in MUD transplants for SAA.[15]

Unfortunately many of these studies have not been confirmed, and the general feeling among transplant teams, at least in Europe, is that MUD BMT are unsuccessful in SAA: to support this position is the fact that there are less than 70 transplants from non HLA-identical donors in the EBMT database. This is less then 1/10th of the transplants from HLA-identical siblings, even though there are twice or even three times as many candidates without an identical sibling donor. Thus transplant teams are still unwilling to graft SAA patients who lack HLA-identical sibling donors. This is quite reasonable if one considers the good results obtained with IS and growth factors.[16]

Cord blood (CB) banks are being established in Europe and in the USA with the goal of making CB stem cells available for unrelated transplants: over 50 CB transplants have been performed in HLA-matched siblings, proving that CB can produce permanent engraftment and perhaps induce less acute GvHD than bone marrow.

Non transplant approaches

Immunosuppressive treatment. Immunosuppressive treatment (IS) has evolved over the past two decades, and results have improved significantly in all categories of patients: overall 5-year actuarial survival was 43% for patients treated between 1970 and 1984, and 66% for those treated thereafter (p=0.0001). High-dose methylprednisolone[21] is not used as a single agent any longer. *Antilymphocyte globulin* (ALG) continues to be the most frequently employed agent, although cyclosporine is increasingly being used, both in conjunction with ALG and as a single agent. The French trial compared CyA alone versus ALG alone as first-line SAA therapy.[17] Patients who did not respond were crossed over to the other arm; for this reason results are difficult to interpret, although there seems to be an advantage for patients treated initially with CyA versus those treated initially with ALG. There have been two additional prospective randomized trials designed to optimize immunosuppressive treatment (Table 1). The German trial looked at the effect of adding CyA to a standard regimen of ALG plus methylprednisolone (6MP):[18] there

Table 1. Comparison of two randomized multicenter trials designed to optimize IS treatment of SAA.

Trial	Survival	Response
ALG alone vs	54%	34%
ALG+ CyA (German)[18]	61%	65%
ALG alone vs	65%	40%
ALG+ androgens (EBMT)[19]	73%	57%

Abbreviations: ALG= antilymphocyte globulin; CyA= cyclosporin A

was a moderate advantage for patients receiving CyA in addition to ALG+6MP. The EBMT trial[19] examined the effect of adding androgens to a standard regimen of ALG+6MP. One-hundred thirty-four patients entered the trial and a significant advantage in terms of response at 4 months was seen in those receiving androgens. This advantage was exclusively due to increased responses in females with less than 0.5×10^9/L PMN. The conclusion of that study was that androgens improve response rates in females with SAA, though this has not as yet translated into a survival advantage. A meta-analysis of the German and EBMT trials was recently presented at an ASH meeting:[20] response rates were similar in the two control arms (34% and 40% at 4 months) and were improved by the addition of CyA (65%, p=0.04) or androgens (57%, p=0.08). Long-term survival was also comparable in the two control arms (54% and 65%, p=0.5) and was not significantly improved by the addition of CyA (61%) or androgens (73%).

Taken together these trials show that IS regimens can be modified and response rates can be increased; they also show that survival is affected less than response rates, at least in the short term. Finally, they have confirmed that early mortality is dependent on the level of circulating PMN.

Growth factors. Several hemopoietic growth factors have become available for clinical use: G-CSF, GM-CSF, EPO, IL3 and, recently, stem cell factor. The myeloid growth factors have been shown to improve the leukocyte counts of patients with chemotherapy-induced cytopenia[22-23] and to accelerate leukocyte recovery after marrow transplantation.[24]

Erythropoietin can correct anemia in subjects with low Epo levels, such as renal failure patients,[25] and can also ameliorate erythropoiesis in some patients with adequate EPO levels (MDS). It was therefore tempting to use growth factors in patients with SAA, and results demonstrated that growth factors alone produce a moderate to significant improvement in the leukocyte count.[26] One prospective randomized trial comparing treatment with ALG alone versus ALG plus GM-CSF was conducted within the EBMT: leukocyte counts were improved in patients receiving growth factors, but responses and overall survival were not affected (unpublished). Interleukin-3 (IL3) was also used without success (unpublished). Recently, IL5 was employed in a pilot study in Germany without success.[27] The EBMT WPSAA conducted a pilot study on G-CSF in conjunction with ALG and CyA, and very encouraging results on early mortality were found.[16] In conclusion, growth factors may be helpful in improving leukocyte counts in patients with SAA; they should not be used alone, but rather in association with other immunosuppressive agents in prospective trials.[28]

BMT vs IS

The Registry of the EBMT Working Party for SAA shows 598 patients have been treated with IS between 1985 and 1994 , and 5-year actuarial survival is 68%. After stratification for PMN (≤ 0.5; >0.5×10⁹/L), 5-year actuarial survival becomes 65% and 70%, respectively. Table 2 outlines survival for patients further stratified for age (0-19; 20-39, ≥40), with results of HLA-identical BMT reported for comparison. In patients with low PMN counts (≤ 0.5×10⁹/L) BMT and IS yield comparable results, with a slight advantage for BMT, especially in young patients. In those with PMN counts >0.5×10⁹/L IS seems to have an advantage, especially in older patients.

Of course survival is not the only goal: quality of life and secondary effects should also be taken into account. IS patients have a lower quality of hematologic reconstitution, and some patients may require long-term transfusion support, thus remaining exposed to viral infections that

Table 2. Actuarial survival of patients treated between 1985 and 1994 with IS or BMT in Europe, and stratified according to age, and level of PMN count.

| | 5-year actuarial survival | | | |
	age 0-19	age 20-39	age 40+	overall
IS (n=598)				68%
PMN 0.2-0.5x10⁹/L	66%	60%	52%	65%
PMN >0.5x10⁹/L	70%	90%	70%	70%
BMT (n=391)	69%	70%	62%	30%

Abbreviations. IS= immunosuppression; BMT= HLA-identical bone marrow transplantation; PMN= polymorphonuclear cells.

can cause considerable morbidity.

IS patients have a higher risk of developing MDS/leukemia many years after treatment.[29] BMT patients are exposed to a higher risk of infectious complications, and graft rejection, either as true rejection or as late graft failure. Chronic GvHD should also be taken into consideration.

Algorithm for first-line treatment of SAA

Individual treatment should be designed only after the outlined diagnostic algorithm has been followed, and a diagnosis of SAA has been confirmed.

There are three variables that should be considered when designing the treatment strategy for a patient with SAA: 1) availability of an HLA-identical sibling or a syngeneic twin; 2) neutrophil count (PMN); 3) patient's age.

The interactions of these variables are shown in Table 3 and suggest the following guidelines.

Indications for HLA typing

HLA typing of patient and siblings should be performed immediately for those patients under the age of 40. In the absence of a matched sibling, the parents of children under the age of 15 years and with low neutrophil counts should be HLA typed; molecular typing of class II antigens should also be performed to initiate a search for a matched unrelated marrow or unrelated cord blood donor.

Table 3. Therapeutic approach to the patient with an HLA-identical donor. If the PMN count is low (<0.5x10⁹/L), allogeneic BMT is probably the treatment of choice up to the age of 40. In older patients a first course of IS can be administered, and transplant reserved for non responders. If the PMN is higher (≥0.5x10⁹/L), transplant will be first line up to the age of 20 and second line thereafter.

First line treatment	HLA-identical donor	PMN ($\times 10^9$/L)	Age (years)
BMT	yes		< 20
	yes	≤ 0.5	< 40
IS	no		
	yes	> 0.5	≥ 20
	yes		≥ 40

Indications for BMT

Syngeneic BMT will be the first choice in all patients under 60 with an identical twin. They should be prepared with cyclophosphamide at a dose of 200 mg/kg. For older patients a course of ALG followed by the infusion of syngeneic marrow would be best.

Allogeneic BMT is the first-line treatment in patients under 20 with an HLA-identical sibling, irrespective of PMN, or between the ages of 20 and 40 when the PMN is ≤ 0.5×10⁹/L. The regimen for allogeneic BMT should include cyclophosphamide for conditioning and CyA for GvHD prophylaxis. This regimen should not include radiation due to the well-documented long-term side effects. For patients who have been heavily transfused and are therefore highly sensitized, it may be appropriate to include ALG along with the cyclophosphamide.

Indications for IS

IS should be first-line treatment for patients without an HLA-identical sibling and for those over 40 years of age. It may also be considered for patients over 20 years old with an HLA-identical donor and with a high neutrophil count (>0.5×10⁹/L).

Candidates for IS with PMN above 0.5×10⁹/L should all receive CyA at a dose of 5 mg/kg/day, per os, for at least 6 months, after which CyA should not be discontinued abruptly since this may cause a relapse of the aplasia. Survival at 2 years can be expected to be greater than 90%.

Patients with PMN counts ≤ 0.5×10⁹/L should receive ALG 1.5 mL/kg/day on days 1-5, prednisolone 2 mg/kg/day on days 1-5, CyA 5 mg/kg/day p.o on days 1-180, and recombinant human G-CSF 5 ug/kg/day s.c on days 1-90. Survival at 3 years can be expected to be approximatively 92%.[19]

Most centers are at present using Merieux ALG, which is available as horse and rabbit ALG. Horse ALG is used in the first treatment, and rabbit ALG in the second. All trials in Europe are currently being run with Merieux ALG.

Second-line treatment

As illustrated in Figure 1, second-line treatment is considered in patients showing no response after a first course of IS. One hundred days would seem to be an appropriate time for evaluating response. If the patient has an HLA-identical sibling, BMT should be performed. If there is no donor a second course of IS should be given, with a different ALG (rabbit ALG as opposed to horse ALG usually). Androgens may be added to the second IS course, especially in females with an PMN count lower than 0.5×10⁹/L growth factors (G-CSF), even long term, may prove very helpful.

Unrelated marrow transplantation has not produced encouraging results in patients with acquired SAA; they are certainly inferior to what can be achieved at present with IS treatment. An exception may be the very young child (less than 5 years old) with very severe aplasia.

Conclusions

Patients with cytopenia should be diagnosed correctly and treatment started according to one of the ongoing trials. Growth factors should NOT be used alone, but in combination with ALG and CyA in patients with severe aplasia. Cortisone should not be used alone. CyA alone is at present not indicated for patients with very severe aplastic anemia. CyA should not be discontinued abruptly in case of hematologic response, which is often slow and progressive.

HLA typing should be performed up front. Irradiation should NOT be used in the conditioning regimen. The marrow should not be T cell depleted. The marrow cell dose should be as high as possible. CyA should be tapered very slowly even after allogeneic BMT. Unrelated marrow transplant is at present indicated only in very young children who do not respond to IS.

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