ALLOGENEIC STEM CELL TRANSPLANTATION (HSCT) IN CHILDREN WITH SEVERE APLASTIC ANEMIA (SAA).

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Severe aplastic anemia (SAA) was one of the first indications for stem cell transplantation (SCT) with the aim of to repopulating an empty marrow by healthy allogeneic stem cells. In 1972, the first successful engraftment of bone marrow stem cells in SAA was carried out in Seattle (USA) [1]. In Germany (Munich) [2] and Poland (Poznań) [3] the first engraftments were made in 1975 and 1982, respectively. Since then, however, the indication for SCT in SAA has relatively decreased, as shown by Gratwohl et al. [4], from 25 % in 1973 to 5 % in 1997, compared with the increase in leukemias from 13 % to 79 %, over the same period of time.

Though results in alternative - mainly combined immunosuppressive - therapy (IST) for SAA have also distinctly improved, SCT remains the first option in children and young adults who have an available HLA-identical sibling as a donor. In the last 20 years survival after SCT for SAA increased from 52 % to 78 % [5]. In children, survival rates as high as 85 % or more in some centers have been reported [6]. This success was due to more effective conditioning, GVHD prophylaxis and supportive care. The main problems that still remain are graft rejection and - to a lesser degree- chronic GVHD.

Results from donors other than HLA-identical siblings have so far been less encouraging. That is why this therapeutic option is considered as second line treatment for children who do not respond to immunosuppressive therapy.

In 1994, a prospective multicentric German-Austrian study was started to standardize treatment for SAA in chil-}dren, and to compare results of SCT and IST [7]. In this report current results will be shortly summarised.

PATIENTS AND DIAGNOSTIC PROCEDURES

Thirty - five centers in Germany and Austria participated in this study. A total of 122 children with newly diagnosed SAA according to the CAMITTA criteria [8] were investigated between November 1993 and June 1998. Children with congenital disorders (e.g. FANCONI-anemia) or previous treatments were excluded. The diagnosis of SAA was based on bone marrow biopsy in all cases. In addition, conventional cytogenetics was performed in 87 patients, as well as interphase *in situ* hybridisation (FISH) for the detection of monosomy 7 or trisomy was carried out 8 in 46 patients.

STUDY DESIGN (fig. 1)

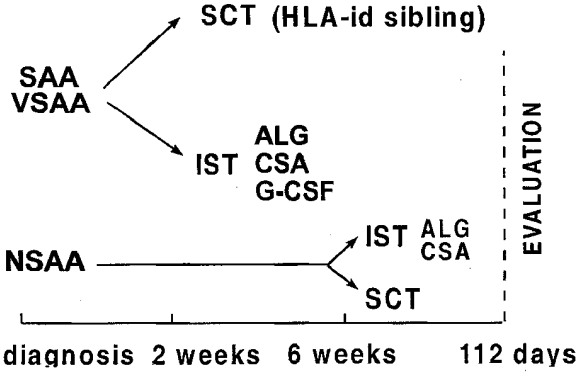

Fig. 1. Study design of SAA94 pilot protocol.

Patients were classified according to their PMN counts in VSAA, SAA and NSAA (see table 1), and the availability of an HLA-identical donor. After a minimal observation time of two weeks in VSAA and 6 weeks in NSAA, those with an available HLA-identical family donor were prepared for SCT, while the rest received combined immunosuppressive therapy.

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SCT-GROUP

37/122 patients had an HLA-identical family donor. All but two (donor not available, refusal of parents) underwent primary SCT. Conditioning therapy consisted of 50mg/kg/d cyclophosphamide for a total dose of 200 mg/kg in one untransfused patient. All others received in addition ATG (horse, LTD. Merieux) 0.75 ml/kg/d. (approximately 15 mg/kg/d) for 4 days. Unmanipulated bone marrow stem cells were given 24 h after the last dose. GVHD prophylaxis consisted of cyclosporine A (CSA) and a short course of methotrexate (MTX) (15 mg/m^2 /d +1, 10 mg ^{2} /d + 3, +6, +11) CSA dosage was adjusted to their blood levels and was discontinued after d+ 100, except in case of GVHD or mixed chimerism.

IST GROUP

The remaining 87 patients received combined immunosuppressive treatment consisting of ATG (horse; Ltd Merieux) 0.75 ml/kg/d (approximately 15 mg/kg/d) for 8 days. For prophylaxis of acute adverse allergic reactions and serum sickness prednisolone 1 mg/kg/d was given for 28 days and then discontinued. In addition, all patients received CSA 5 mg/kg/d, adjusted to their blood levels orally for at least 6 months. In VSAA and SAA, G-CSF (filgrastim Ltd AMGEN) was given in a dosage of 5 μg/kg/d from day 1 to day 28, and then increased to 10 μg/kg/. until day 56 in case of continuous PMN counts below 1.5 G/l. In responders, G-CSF was tapered off the same dose being given with increasing intervals. Response criteria are shown in table 2.

Tab. 2.

Immunosuppressive therapy (IST) **Response criteria**
Complete Response (CR) Hb > 12g/dl PMN > 0.5 G/l platelets > 100 G/l
Partial Response (PR) PMN > 0.5 G/l reticulozytes > 30 G/l transfusion independency
Sole PMN-Response to G-CSF PMN > 0.5 G/l transfusion dependency of platelets and/or red cells

SUPPORTIVE THERAPY

All patients were transfused with leukocyte depleted irradiated (with 20 Gy) and CMV -IgG - negative blood products. Routine antifungal prophylaxis consisted of oral itraconazol and inhalation with an amphotericin B solution. Fever patients received intravenous broad spectrum antibiotics, non-absorbable antibiotics were not given. During periods of severe neutropenia reverse isolation and strict hand desinfection were recommended.

STATISTICS

Kaplan-Meier plots were used to calculate the actuarial probability of survival, the probability of relapse and that of clonal disease, and the kinetics of response. The logrank test was used for comparison of Kaplan-Meier curves. Contingency tables, CHI-square test and Fisher's exact test were used to investigate possible risk factors.

PATIENT CHARACTERISTICS

In 9 children the disease was associated with eareier hepatitis (1 hepatitis B, 1 hepatitis C, 7 with elevated transaminases, but negative for hepatitis A, B, C). All other cases must be classified as "idiopathic". The median age of the whole group was 9.5 y. (0.9 – 16.9 y.) There was a slight, but not significant difference in age, gender, severity of SAA and interval between diagnosis and treatment between the two groups treated with SCT and IST resp. (see table 3).

Tab. 1.

Criteria in SAA (according to Camitta (8))
VSAA (= very severe aplastic anemia) PMN count < 0.2 G/l
SAA (= severe aplastic anemia) PMN count < 0.5 G/l
NSAA (= non severe aplastic anemia) PMN count > 0.5 G/l

OVERALL SURVIVAL

Probability of survival of both groups were comparable in children treated with SCT (86 %) and IST (85 %), resp. eclively (see fig. 2).

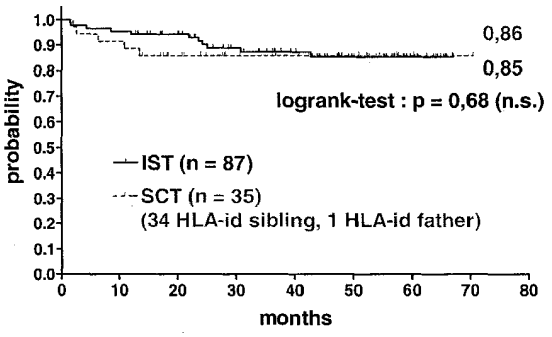

Fig. 2. Probability of survival after IST and BMT.

PRIMARY SCT

Thirty of 35 children undergoing primary SCT survived. Thirty - three were grafted from their HLA-identical siblings, one from her identical twin, and one girl from her phenotypical identical father. The median cell dosage was 3.14 x 10^8 mononuclear cells /kg (1.3-5.4 x 10^8mononuclear cells /kg), two children (6 %) died early after transplant, one of aspergillosis, the other from heart failure. Twenty -six evaluable patients showed stable engraftment. Five patients (15 %) rejected the graft 1 - 49 months (median 6 months) after SCT, one of those having been successfully regrafted. Two children died from severe GVHD after 2nd or 3rd SCT resp. The remaining two patients showed autologous recovery when treated with CSA and G-CSF. There was no difference in age, number of previously given blood products or interval between diagnosis and SCT between the successfully grafted patients and those who rejected the graft.

Severe acute GVHD grade IV occurred in two patients, one of them - the girl grafted from her father - died from extended chronic GVHD and recurrent pneumonia. The other patient developed chronic GVHD involving the liver and gut. With his liver and gut problems resolved, he now suffers from a chronic lung disease, and still needs immunosuppressive treatment. The median age at diagnosis was significantly lower ($p < 0.01$) in surviving patients (n =30, median 8.8 y., range 2.2-15.8 y. vs n= 5, median 15 y. ,range 10.3 –15.4 y.)

PRIMARY IMMUNOSUPPRESSION

Two patients died early because of cranial hemorrhage and severe infection resp. Twelve (14 %) of the remaining 85 patients reached CR at day 112, 29 patients (34 %) at day 180. 61 patients (72 %) were transfusion-independent at this time. At 18 months 35 (47 %) of the evaluable 75 patients reached CR, 54 (75 %) were free of transfusion. Unexpectedly, patients with VSAA showed the best response to IST. After 12 months 42 of 50 children with VSAA showed transfusion independency, and 26 of them (52 %) achiered complete response.

Twenty - four patients were still transfusion dependent at day 112, but responded with their PMN counts. Only four patients did not achieve PMN counts above 500 G/l though high doses of G-CSF, all of them died from severe infection. A trial of the second immunosuppressive treatment in four patients (in three cases with ATG and, in one patient with cyclophosphamide) was not successful, all of them remained transfusion dependent.

In addition to non-response, main problems in the IST-group were relapse and evolution of a clonal disease. Seventeen of the 72 responders relapsed, the probability of relapse being 28 % after 67 months (see fig. 3). Nine patients achieved a second response after reinduction of CSA.

In 13 patients, a clonal disease developed 2 – 35 months after beginning of IST with a probability of 18 % at 67 months. Four patients progressed to AML, four to MDS after partial or complete response to IST. In the remaining 5 patients chromosomal abnormalities were observed without evidence of malignant evolution (fig. 4)

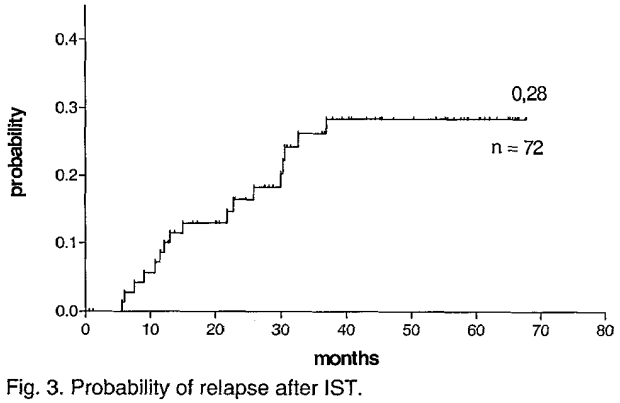

Fig. 3. Probability of relapse after IST.

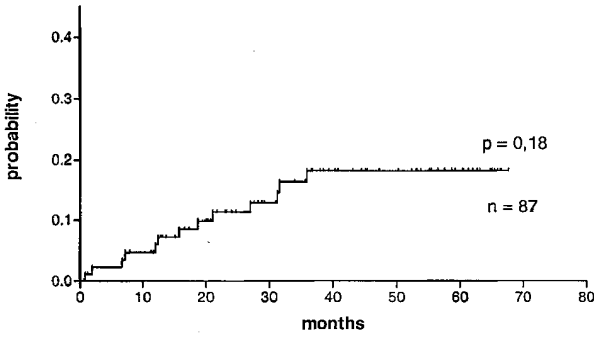

Fig. 4. Probality of clonal disease after IST.

SECONDARY SCT

Thirteen patients received secondary SCT because of the failure of IST (n= 7), therapy resistant relapse (n = 3) and / or clonal disease (1 AML, 2 MDS). One child was grafted from HLA-id. sibling , the other 12 from alternative stem cell donors; 4 / 12 received stem cells from family donors, one from a phenotypical identical aunt, one from HLA-1Ag-mismatched mother, and two from haplo-identical parents. The remaining 8 patients were grafted from HLA-identical unrelated donors. The median interval from diagnosis to SCT was 22 months (range 10 – 45 months). A conditioning regimen was not strictly defined for participants in the study. The recommended regimen, according to the protocol used in SEATTLE (9), consisting of low dose TBI (2 x 2 Gy), cyclophosphamide (200 mg/kg) and ATG was employed in three patients, all of them survive.

The remaining patients received various regimens depending on the status of the disease, HLA-incompatibility of a donor or manipulation of the graft. Seven patients were irradiated with higher TBI- doses (7.5 – 12 Gy), partly in combination with T-Cell- depletion (n = 1) or CD 34 positive stem cell selection (n = 3). Two children with MDS and monosomy 7 received Busulfan- based regimens.

Two patients grafted from their haplo-identical parents died after successful engraftment from severe virus infection (1 from CMV, 1 from EBV); two further patients died, one after graft failure from apergillosis and, the other from acute lung emboly after 10 months. Though a total of 8 patients survived, there were distinctly more complications in SCT from alternative donors compared with the situation in HLA-sibling donors.

Tab. 3. Clinical characteristics of 122 children with aplastic anemia.

	All	BMT	IST
Number of patients	122	35	87
Median age (range) (yrs)	9.5 (0.9 - 16.9)	10.0 (2.2 - 15.8)	9.1 (0.9 - 16.9)
Sex : male / female	67 / 55	14 / 21	53 / 34
Severity VSAA	70	18	52
SAA	43	15	28
NSAA	9	2	7
Median duration (range)	28 (3 - 272)	48 12-272	23 (3-168)

DISCUSSION

Results in SCT from HLA-id. sibling donors have essentially been improved over the last decade, especially in young patients with SAA. [10]. This success is mainly due to better supportive care and decreased incidence of acute and chronic GVHD [10]. In our reported series only two adolescent patients suffered from severe Gr IV acute GVHD followed from chronic GVHD

In addition, the dominating problem of graft rejection in patients with SAA could be significantly reduced, though there is still a rate of about 10 % in all reported studies [11]. In our patients a rejection rate of 15 % was observed, in two of them with a fatal outcome after repeated SCT. In case of late

rejection a second course of CSA may be attempted.

Thus, for primary SCT the combination of cyclophosphamide and ATG seems to be an optimal regimen, with radiotherapy avoided to reduce the risk of a second malignancy [12].

Beside secondary malignancy, late effects after SCT seem to be rare with the exception of the sequelae of chronic GVHD. In our earlier patients we observed an increased risk of a progressive lung disease [13]. In the cases presented here, the patient with chronic GVD suffers from deteriorating bronchiolitis obliterans.

Most children with SAA, however, lack an available sibling donor; in our series 86/ 122 (70 %). In this situation a combined immunosuppressive therapy is recommended
[14]. Results of IST could also be improved due to a better supportive care and possibly due to early administration of G-CSF. In our patients, the overall response was 77 %, and was even better in VSAA, which contradicts the earlier results of Locasciulli et al. [15]. As survival in both treatment strategies- SCT or IST- are comparable, there is already discussion under way whether IST should be preferred as a front line therapy [16].

However, two problems in IST remain unresolyed: the high rate of relapse and the risk of a clonal disease, already observed by Speck and his group 1990 [17] The role of G- CSF in the evolution of a clonal disease, especially monosomy 7 remains controversial [18] Careful diagnostic procedures are essential at the beginning of the disease to rule out other causes of aplasia (e.g. hypoplastic MDS). Regular screening – especially for monosomy 7 – is necessary in the course of SAA after IST. So far, no clonal diseases of myeloid origin have been observed after SCT [12].

Results from alternative donors in SAA are far worse than these from HLA- id. siblings. In 1999, J.Hows reported survival rates of 40% in SCT from unrelated donors and 45 % from alternative family donors at 3 y, based on various registry data [19]. A better survival of 54 % was reported by Margolis et al [20] in children and young adults when a very intensive conditioning regimen was need including FTBI with 12-14 Gy. The best results were achieved in two Japanese reports with higher than 80 % survival rate in smaller series of children [21, 22].

In our study, secondary SCT from alternative donors was recommended urgently in cases of non-response to G-CSF with granulocyte counts., in a manifest clonal disease, continuous transfusion dependency or therapy resistant relapse. In the latter cases, the decision for SCT was often delayed as the quality of life in those patients was frequently rather good. Thus, the interval from diagnosis to SCT varied considerably. A conditioning regimen was not strictly defined. In cases of AML/ MDS a more intensive regimen were used. Comparable results were achieved in patients conditioned with low dose FTBI and cyclophosphamide and those conditioned with higher doses of irradiation and T-cell depletion of the graft or CD 34 selected stem cells). The two children transplanted from their haplo-id. parents died from infection.

Possibly, we would have achieved better results and fewer complications, if SCT had been performed earlier in the course of the disease. Second trials with common immunosuppressants had been not successful in our patients.

In summary, SCT is the best option for children with SAA , if an HLA-id. sibling as a donor is available. In the remaining children a combined immunosuppressive treatment should be given with careful monitoring of a clonal disease. There is enough evidence that ATG and cyclophosphamide are an acceptable conditioning regimen for children with an HLA- id. sibling as a donor. The addition of irradiation seems to be necessary in the cases of an alternative donor, the role of T-cell depletion has not yet been clearly defined. The prognosis for children with resistant SAA without an suitable donor remains grave, SCT from an haplo-id donor seems to be no successful alternative. It may be that the development of new potent immunosuppressive drugs is a perspective for those patients.

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