Brief report

Immunosuppressive therapy for aplastic anemia in children: a more severe disease predicts better survival

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Severe acquired aplastic anaemia (SAA) is a life-threatening disease characterized by pancytopenia and hypoplastic bone marrow. Autologous T lymphocytes are thought to cause bone marrow failure by immune-mediated excessive apoptosis of stem and progenitor cells. The disease is subclassified into a severe (neutrophil count, $> 0.2 \times 10^9/L$ [$> 200/\mu$ L]) and a very severe ($< 0.2 \times 10^9/L$ [$< 200/\mu$ L])

(vSAA) form. We report the results of a prospective multicenter trial with a combined immunosuppressive regimen of cyclosporin A (CSA), anti-thymocyte globulin (ATG) and, in cases with neutrophil counts fewer than 0.5 × 10⁹/L (< 500/ μ L), granulocyte colony-stimulating factor (G-CSF) for treatment of SAA in children. Children with vSAA showed a higher rate of complete response than did

children with SAA (68% versus 45%; P = .009), as well as better survival (93% versus 81%; P < .001). Thus, in children with SAA a more severe disease stage at diagnosis indicates a favorable outcome with immunosuppressive therapy. (Blood. 2005;106:2102-2104)

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Introduction

A more severe form of a given disease negatively affects outcome in almost all human diseases. This seemed likely to apply to severe acquired aplastic anaemia (SAA), the pathophysiology of which is characterized by immune-mediated bone marrow failure.¹ A predominant oligoclonal immune response² by autologous T lymphocytes was shown to cause excessive apoptosis in stem and progenitor cells.^{3,4} The molecular target of this T-cell response is still unknown, although in vitro a T-cell–mediated inhibition of aneuploid hematopoietic cells was demonstrated in patients with myelodysplastic syndrome.⁵

Immunosuppressive therapy (IST)⁶ seems to be the appropriate treatment for an autoimmune disease, but tissue replacement by bone marrow transplantation (BMT) is still the treatment of first choice^{7,8} because there have been 20% to 30% IST nonresponders, a significant proportion of patients with subnormal blood counts⁹ and a high risk of relapse^{10,11} and clonal disease.^{12,13} Because of the long latency of response to IST, identification of predictors of response and survival would be helpful to assign patients to the appropriate regimen.

In children very SAA (vSAA) is predominant (> 60%). Patients with vSAA are especially prone to develop life-threatening infections. As most of those high-risk patients lack an HLA-identical

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A complete list of the members of the German/Austrian Aplastic Anemia Working Group appears in the "Appendix."

sibling donor (matched sibling donor; MSD), searching for a matched unrelated donor (MUD) was recommended, despite that BMT from a MUD is still complicated by a high risk of graft rejection and graft-versus-host disease. This recommendation was well founded on the basis of a previous retrospective analysis¹⁴ in which the outcome for children with vSAA was the worst with IST consisting of antithymocyte-globulin (ATG) plus androgens and/or steroids (probability of survival: vSAA, 37%; SAA, 56%).

Combined IST with ATG and cyclosporin A (CSA) has been shown to be superior to ATG alone or in combination with androgens in adults,^{15,16} and the addition of granulocyte colonystimulating factor (G-CSF) to the treatment regimen was able to improve granulocyte recovery.^{17,18}

We conducted a prospective multicenter trial comparing combined IST plus G-CSF and BMT¹⁹ to identify prognostic factors for response to therapy and survival.

Study design

Two hundred and thirteen patients newly diagnosed with SAA younger than the age of 17 years in 53 centers in Germany and Austria were included in

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M.F., U.R., C.N., G.J.-S., W.F., A.B., and C.B.-G. conceived the study, designed the approach, and interpreted the data. M.F. wrote the paper. A.F. performed and interpreted the statistical analysis. I.B. reviewed the bone marrow biopsies to establish the correct diagnosis.

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the study between November 1993 and December 2001 (Table 1). The treatment protocol was approved by the local Ethics Committees. Informed consent was given by parents. For this study approval was obtained from the institutional review board of the Medical Department of the Ludwig-Maximilians University of Munich. Fanconi anemia was excluded by chromosomal fragility test. The diagnosis of SAA was based on morphology and blood counts.²⁰ Zytogenetics and/or fluorescence in situ hybridization (FISH) analysis for monosomy 7 and trisomy 8 were performed in 158 of 213 patients at diagnosis. Clonal disease was detected during follow-up in 15 patients who received IST (8 cases with monosomy 7); in 3 of them the aberration was already present in the initial bone marrow (BM).

By biologic selection depending on the availability of an MSD, patients were assigned to either the BMT (n = 67) or the IST group (n = 146). Sixty-two patients received BMT after a conditioning treatment with ATG (horse; Sangstatt/Genzyme, Neu Isenburg, Germany) (0.75 mL/kg body weight [BW] for 4 days) and cyclophosphamide (50 mg/kg BW for 4 days). In the remaining 5 patients, 3 with SAA and 2 with vSAA, parents refused BMT. One hundred fifty-one children (151; 5 with an MSD) were treated with combined IST, including ATG (horse; 0.75 mL/kg BW for 8 days; Sangstatt/Genzyme), CSA (5 mg/kg BW, adjusted to blood levels), prednisolone (1-2 mg/kg tapered until day 28), and, in cases with neutrophil count (polymorphonuclear leukocytes; PMNs) fewer than $0.5 \times 10^9/L$ [500/µL], with G-CSF (5 µg/kg BW) in addition (8 SAA patients did not receive G-CSF). After 28 days the G-CSF dose was increased to 10 µg/kg when PMNs were fewer than $1.5 \times 10^9/L$ (1500/µL), whereas, when PMNs were more than $1.5 \times 10^9/L$ (1500/µL), G-CSF was slowly tapered.

Response to IST and survival were evaluated on days 28, 42, and 112, at 6 months, and every 6 months afterward. Complete response (CR) was registered when hemoglobin reached age-adjusted normal values, platelet count was more than $100.0 \times 10^9/L$ (>100 000/µL), and PMNs were more than $1.5 \times 10^9/L$ (>1500/µL). All criteria had to be fulfilled. Partial response (PR) was diagnosed in patients with transfusion independency, when reticulocyte count was more than $30.0 \times 10^9/L$ (>30 000/µL), and PMNs were more than $0.5 \times 10^9/L$ (>500/µL) above baseline. Minimum projected follow-up was 2 years; median follow-up at time of analysis was 50 months (range, 1-116 months). The analysis was based on intention to treat.

Results and discussion

In 137 (64%) of 213 patients vSAA was diagnosed. Within the BMT group, patients with vSAA (n = 40) and with SAA (n = 27) reached comparable survival rates (5-year survival rate: vSAA, 89%; 95% confidence interval [CI], 80%-99%; SAA, 96%; 95% CI, 89%-100%).

Ninety-seven vSAA patients without MSD were selected to receive IST. In this high-risk group the 5-year survival rate was



Observation time (mo)

Figure 1. Survival rate after IST for children with vSAA (93% after 5 years; 95% CI, 88%-98%) and SAA (81% after 5 years; 95% CI, 69%-93%; P <.001).

93% (95% CI, 88%-98%), a clear improvement compared with previously published data. Surprisingly, patients with SAA (n = 49) in the IST group showed a lower 5-year survival rate, namely 81% (95% CI, 69%-93%; *P* of log-rank test < .001) (Figure 1). This was due to a better response to IST by patients with vSAA, who reached CR in 69% (95% CI, 60%-78%), compared with 44% of patients with SAA (95% CI, 30%-58%; *P* = .004, Fisher exact test) (best response 1 year after start of treatment). There was a tendency for better relapse-free survival for patients with vSAA compared with patients with SAA (80% versus 67% at 5 years; 95% CI, 70%-91% versus 51%-83%) (*P* < .065, log-rank [LR] test). Age and time to therapy were different between SAA and vSAA (Table 1). In a multivariate Cox regression model adjusted for age the interaction between severity and intended therapy proved to be relevant. Time to therapy had no prognostic relevance there.

After 6 months, response to IST strongly predicted further survival in all patients then alive, with a 5-year survival of 100% in CR, 97% in PR (95% CI, 92%-100%), and 67% in patients with no response (NR) (95% CI, 48%-86%; *P* value of LR test: CR and PR versus NR < .001, CR versus PR = .271).

We conclude that in children with AA, disease severity no longer predicts inferior survival, when no MSD is available. In vSAA, treatment with combined IST and G-CSF converts the former bad prognosis factor severe granulocytopenia ($< 0.2 \times 10^{9}/L$ [$< 200/\mu$ L]) into an indicator of excellent response and survival. Part of this progress may also be due to improved supportive care. Thus, early BMT from MUD is no longer recommended in this good-prognosis group.

Table 1. Patient characteristics in childre	treated with IST or BMT features	or severe aplastic anemia
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Table 1.1 allent ondrablenolido in onnaren ricaled with or of bint for severe aplasite anemia						
	All patients	Patients with SAA	Patients with vSAA	Р		
No. patients	213	76	137			
Age, median, y (range)	8.9 (0.91-16.95)	10.43 (1.42-16.93)	8.06 (0.91-16.95)	.010*		
Sex, no. (%)				.146†		
Male	125 (59)	50 (66)	75 (55)			
Female	88 (41)	26 (34)	62 (45)			
Etiology, no. (%)				.874		
Idiopathic	171 (80)	63 (83)	108 (79)			
Toxic	9 (4)	3 (4)	6 (4)			
After hepatitis	18 (9)	6 (8)	12 (9)			
Postviral infection	15 (7)	4 (5)	11 (8)			
Interval between diagnosis and treatment, median, mo (range)	0.90 (0.03-8.94)	1.26 (0.20-8.94)	0.92 (0.03-8.21)	.002§		

*Unpaired t test.

†Fisher exact test.

‡χ² test.

§Mann-Whitney U test.

After 6 months, response to IST predicted further survival.²¹ Nonresponders at that time should proceed to MUD BMT. However, the long latency period of recovery after IST disqualifies response to therapy as a tool for early therapeutic decisions.

A different treatment approach was chosen by a Japanese group. They administered androgens in addition to ATG, CSA, and G-CSF, with comparable results.²² In their study no difference in outcome between SAA and vSAA was observed, which was likely due to a number of cases with androgen-responsive congenital bone marrow failure hidden in the SAA group. Because of the excellent outcome in our study and the severe side effects of androgens, we strongly recommend that these drugs should be omitted from SAA treatment in children.

Our findings clearly suggest that especially in children with vSAA the immune system plays the key role in the pathomechanism of bone marrow failure, and the per se healthy stem cell compartment is able to compensate for the loss of stem cells as soon as the T-cell-mediated destruction is interrupted.²³ Severe granulocytopenia at diagnosis may therefore serve as a surrogate marker for an immune-mediated disease instead of a primary stem cell disorder.

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Appendix

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References

- Young NS, Maciejewski J. The pathophysiology of acquired aplastic anemia. N Engl J Med. 1997; 336:1365-1372.
- Risitano AM, Maciewski JP, Green S, et al. In-vivo dominant immune responses in aplastic anemia: molecular tracking of putatively pathogenic T-cell clones by TCR beta-CDR3 sequencing. Lancet. 2004;364:355-364.
- Killick SB, Cox CV, Marsh JCW, et al. Mechanisms of bone marrow progenitor cell apoptosis in aplastic anemia and the effect of anti-thymocyte globulin: examination of the role of the Fas-Fasinteraction. Br J Haematol. 2000;111:1164-1169.
- Young NS. Hematopoietic cell destruction by immune mechanisms in acquired aplastic anemia. Semin Hematol. 2000;37:3-14.
- Sloand EM, Kim S, Führer M, et al. Fas-mediated apoptosis is important in regulating cell replication and death in trisomy 8 hematopoietic cells but not in cells with other cytogenetic abnormalities. Blood. 2002;100:4427-4432.
- Speck B, Gluckman E, Haak HL, van Rood JJ. Treatment of aplastic anemia by antilymphocyte globulin with and without allogeneic bone-marrow infusions. Lancet. 1977;2:1145-1148.
- Bacigalupo A, Brand R, Oneto R, et al. Treatment of acquired severe aplastic anemia: bone marrow transplantation compared with immunosuppressive therapy: The European Group for Blood and Marrow Transplantation experience. Semin Hematol. 2000;37:69-80.
- Ades L, Mary J-Y, Robin M, et al. Long-term outcome after bone marrow transplantation for severe aplastic anemia. Blood. 2004;103:2490-2497.
- Viollier R, Passweg J, Gregor M, et al. Qualityadjusted survival analysis shows differences in outcome after immunosuppression or bone marrow transplantation in aplastic anemia. Ann Hematol. 2005;84:47-55.
- 10. Frickhofen N, Heimpel H, Kaltwasser JP, Schre-

zenmeier H. Antithymocyte globulin with or without cyclosporine A: 11-year follow-up of a randomized trial comparing treatments of aplastic anemia. Blood. 2003;101:1236-1242.

- Führer M, Burdach S, Ebell W, et al. Relapse and clonal disease in children with aplastic anemia (AA) after immunosuppressive therapy (IST): the SAA 94 experience. German/Austrian Pediatric Aplastic Anemia Working Group. Klin Padiatr. 1998;210:173-179.
- Kojima S, Ohara A, Tsuchida M, et al. Risk factors for evolution of acquired aplastic anemia into myelodysplastic syndrome and acute myeloid leukaemia after immunosuppressive therapy in children. Blood. 2002;100:786-790.
- Socie G, Rosenfeld S, Frickhofen N, Gluckman E, Tichelli A. Late clonal diseases of treated aplastic anemia. Semin Hematol. 2000;37:91-101.
- Locasciulli A, van't Veer L, Bacigalupo A, et al. Treatment with marrow transplantation or immunosuppression of childhood acquired severe aplastic anemia: a report from the EBMT SAA Working Party. Bone Marrow Transplant. 1990;6: 211-217.
- Frickhofen N, Kaltwasser JP, Schrezenmeier H, et al; for the German Aplastic Anemia Study Group. Treatment of aplastic anemia with antilymphocyte globulin and methylprednisolone with or without cyclosporine. N Engl J Med. 1991;324: 1297-1304.
- Marsh J, Schrezenmeier H, Marin P, et al. Prospective randomized multicenter study comparing cyclosporine alone versus the combination of anti-thymocyte globulin and cyclosporine for treatment of patients with nonsevere aplastic anemia: a report of the European Blood and Marrow Transplant (EBMT) Severe Aplastic Anaemia Working Party. Blood. 1999;93:2191-2195.
- 17. Bacigalupo A, Broccia G, Corda G, et al; for the European Group for Blood and Marrow Transplantation (EBMT) Working Party on SAA. Anti-

lymphocyte globulin, cyclosporine, and granulocyte colony-stimulating factor in patients with acquired severe aplastic anemia (SAA): a pilot study of the EBMT SAA Working Party. Blood. 1995;85:1348-1353.

- Bacigalupo A, Bruno B, Saracco P, et al; for the European Group for Blood and Marrow Transplantation (EBMT) Working Party on Severe Aplastic Anemia and the Gruppo Italiano Trapianti di Midolio Osseo (GITMO). Antilymphocyte globulin, cyclosporine, prednisolone, and granulocyte colony-stimulating factor for severe aplastic anemia: an update of the GITMO/EBMT study on 100 patients. Blood. 2000;95:1931-1934.
- Führer M, Bender-Götze C, Ebell W, Friedrich W, Kohne E. Aplastic anemia therapy: aims and strategy of the Pilot Protocol SAA 94. Klin Päd. 1994;206:289-295.
- Camitta BM, Rappeport JM, Parkman R, Nathan DG. Selection of patients for bone marrow transplantation in severe aplastic anemia. Blood. 1975;45:355-363.
- Rosenfeld S, Follmann D, Nunez O, Young NS. Antithymocyte globulin and cyclosporine for severe aplastic anemia: association between hematologic response and long-term outcome. JAMA. 2003;289:1130-1135.
- 22. Kojima S, Hibi S, Kosaka Y, et al; for the Japan Childhood Aplastic Anemia Study Group. Immunosuppressive therapy using antithymocyte globulin, cyclosporine, and danazol with or without human granulocyte colony-stimulating factor in children with acquired aplastic anemia. Blood. 2000;96:2049-2054.
- Maciejewski JP, Kim S, Sloand E, Selleri C, Young NS. Sustained long-term hematologic recovery despite a marked quantitative defect in the stem cell compartment of patients with aplastic anemia after immunosuppressive therapy. Am J Hematol. 2000;65:123-131.