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Autologous stem cell transplantation in juvenile idiopathic arthritis : regaining immunological tolerance and arresting disease progression

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Chapter 3

Autologous stem cell transplantation for refractory juvenile idiopathic arthritis: analysis of clinical effects, mortality and transplant related morbidity

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Abstract

Objective. To evaluate the safety and efficacy of autologous stem cell transplantation (ASCT) for refractory juvenile idiopathic arthritis (JIA).

Methods. Retrospective analysis of follow up data on 34 children with JIA who were treated with ASCT in nine different European transplant centers. Rheumatological evaluation employed a modified set of core criteria. Immunological reconstitution and infectious complications were monitored at three months intervals after transplantation.

Results. Clinical follow up ranged from 12 to 60 months. Eighteen of the 34 patients (53%) with a follow up of 12 to 60 months achieved complete drug-free remission. Seven of these patients had previously failed treatment with anti-TNF. Six of the 34 patients (18%) showed a partial response (ranging from 30-70% improvement) and seven (21%) were resistant to ASCT. Infectious complications were common. There were three cases of transplant related mortality (9%) and two of disease related mortality (6%).

Conclusions. ASCT in severely ill patients with JIA induces a drug-free remission of the disease and a profound increase in general wellbeing in a substantial proportion of patients, but the procedure carries a significant mortality risk. The following adjustments are proposed for future protocols: (1) elimination of total body irradiation from the conditioning regimen; (2) prophylactic administration of antiviral drugs and intravenous immunoglobulins until there is a normal CD4+ T cell count.

Introduction

Juvenile idiopathic arthritis (JIA) is the most common rheumatic disease in childhood and a major cause of disability. Although the overall prognosis for most children with chronic arthritis is good, in 5-10% of children with the systemic and polyarticular onset forms, the disease is refractory to conventional treatment, which comprises combinations of non-steroidal anti-inflammatory drugs (NSAID) and immunosuppressive drugs such as methotrexate (MTX) and corticosteroids (1-5). In such cases, there is considerable morbidity from severe joint destruction, growth retardation, psychosocial morbidity, and various adverse effects from long-term treatment with immunosuppressive drugs. Mortality in the whole group of systemic onset children is estimated to be 2-4% (6). The major causes of mortality relate to drug toxicity, intercurrent infection, amyloidosis (7), and the evolution of disease to conditions overlapping with other rheumatic diseases, such as systemic lupus erythematosus or scleroderma (8).

Recently, the introduction of anti-tumour necrosis factor α (TNF α) therapy appeared to have a major impact on outcome of children with polyarticular JIA who were unresponsive to methotrexate, with a persistent response in up to 80% (9). However, in active systemic onset JIA this treatment seems less effective (10-12). Another new treatment approach for patients with severe autoimmune disease involves intense immunosuppression followed by autologous haematopoietic stem cell transplantation (ASCT) (13-17). The rationale of this treatment is based on the concept of intense immunosuppression, with subsequent regeneration of naïve T lymphocytes derived from reinfused haematopoietic progenitor cells. The European League Against Rheumatism (EULAR) and the European Group for Blood and Marrow Transplantation (EBMT) have published guidelines on inclusion criteria, the conditioning regimen, and manipulation of the graft (18,19). Since 1997, ASCT has been applied as an experimental procedure in a substantial group of children with refractory polyarticular and systemic JIA (20-23). The majority of these patients (n = 41) can be identified in the registry of the EBMT. Recently we published a short report on the general outcome of 31 of these children (24). The reported mortality of ASCT in JIA is a major concern.

We report here a more detailed survey of the follow up of 34 of the registered JIA patients who were treated with ASCT in nine different European centers. We evaluate the safety and efficacy of ASCT in this severely ill patient group, with specific attention to infectious complications and transplant related mortality. New adjustments in the transplantation protocol are proposed.

Methods

Patient selection.

We present data on 34 of the 41 JIA patients who received ASCT, identified in the EBMT registry. The patients received their transplants in nine different paediatric transplantation centers in Europe (Table 1). For seven patients registered at the EBMT, the data were not detailed enough to allow follow up evaluation.

Assessment of efficacy.

For rheumatological follow up we used the core set criteria described by Giannini and adopted by the Paediatric Rheumatology International Trials Organization group (PRINTO) were used (25-29). The core set consists of:

- 1) physician's global assessment of overall wellbeing (visual analogue scale, VAS);
- 2) parent/patient's assessment of pain (VAS);
- 3) functional disability (child health assessment questionnaire, CHAQ);
- 4) number of joints with active arthritis;
- 5) number of joints with limited range of motion (paediatric EPM-ROM scale);
- 6) erythrocyte sedimentation rate (ESR) (27-29).

The evolution of the disease in the patients was analysed by collecting the outcome of the core set of variables as recorded before stem cell collection and at three months intervals after transplantation. Owing to the retrospective nature of this study, not all core set criteria were available from every time point for every child; thus Giannini's criteria for improvement were adapted. To meet the definition of improvement at a certain time point, patients had to have a persistent 30% or more improvement from baseline in at least three of the available variables (instead of three of six variables according to Giannini). Partial responders were thus defined as patients with a persistent 30%, 50% or 70% improvement from baseline in at least three response variables. They could also have worsening of 30% in no more than one of the available variables (instead of no more than one out of six variables according to Giannini).

As no criteria for complete clinical remission in JIA have been defined, the American College of Rheumatology (ACR) criteria for complete remission in rheumatoid arthritis (RA) were used (30). Full responders were defined as patients in complete drug-free remission, fulfilling at least 5 or more of the following requirements for the last two consecutive months: morning stiffness for less than 30 minutes, no fatigue, no joint pain, no joint tenderness or pain on motion, no soft tissue swelling in joints or tendon sheaths, and ESR <20 mm/h.

A relapse was defined as a persistent worsening of 30% or more in three of the available core set criteria variables and a minimum of two active joints. There could also be improvement of 30% or more in no more than one of the six response variables. Flare up of disease was defined as a transient worsening of 30% or more in three of the available core set criteria variables and a minimum of two active joints.

Table 1. Clinical characteristics before autologous stem cell transplantation

Case	CP	Sex	Type	Onset	Drug treatment history	Toxicity from antirheumatic drugs	ASCT	FU
1	A	F	Syst	11m	St, MTX, NSAID, CsA, Aza, IVIG	GD, O	6y, 7m	5y
2	A	M	Syst	4y, 7m	St, MTX, NSAID, CsA, Sulfa, Aza, Plaq, Cy	GD, O	10y, 6m	4y
3	A	M	Syst	2y, 6m	St, MTX, CsA, NSAID	GD, O	11y, 5m	4y, 6m
4	A	M	Syst	1y, 2m	St, MTX, CsA, NSAID, Cy, anti-TNF α	GD, O, vertebral fractures, hypertension, hypertonia	4y, 11m	†
5	A	M	Syst	4y, 10m	St, MTX, CsA, NSAID, Aza, IVIG	GD, O	9y, 5m	3y, 6m
6	A	M	Poly	1y, 6m	St, MTX, CsA, Sulfa, Gold im, hydroxy	GD, O, severe Cushing, hypertensive convulsions, proteinuria	9y, 3m	3y
7	A	F	Syst	5y, 2m	St, MTX, CsA, NSAID, Sulfa, Aza, IVIG	GD, O	11y, 3m	4y
8	A	F	Syst	3y, 8m	St, MTX, CsA, NSAID, Aza, hydroxy	GD, O, severe relapsing pneumonitis	14y, 11m	†
9	A	F	Poly	3y, 11m	St, MTX, CsA, NSAID	GD, O	7y, 10m	5y
10	A	F	Syst	7y, 7m	St, MTX, CsA, NSAID	GD, O, liver failure	10y, 7m	2y, 6m
11	A	M	Syst	1y, 8m	St, MTX, CsA, NSAID, anti-TNF α	GD, O	5y, 2m	1y
12	A	M	Poly	2y, 2m	St, MTX, CsA, NSAID, Sulfa	GD, O, severe GI toxicity	8y, 5m	2y
13	A	M	Syst	4y, 1m	St, MTX, NSAID, Sulfa, Auro, Aza	GD, O, severe myelosuppression.	13y, 10m	4y, 6m
14	A	F	Syst	3y, 1m	St, MTX, CsA, NSAID	GD, O	5y, 5m	2y
15	A	M	Syst	3y, 2m	St, MTX, CsA, NSAID	GD, O, Cushing	4y, 3m	†
16	A	M	Syst	5y, 5m	St, MTX, CsA, NSAID, anti-TNF α	GD, O, severe GI toxicity	7y, 9m	1y, 6m
17	A	M	Syst	2y, 10m	St, MTX, CsA, NSAID, anti-TNF α	GD, O	11y, 7m	1y
18	A	M	Syst	1y, 6m	St, CsA, MTX, NSAID	GD, O, hypertension	8y, 2m	1y, 6m
19	A	M	Poly	3y, 1m	St, MTX, NSAID, Sulfa	GD, O	5y, 8m	4y
20	B	M	Syst	8y, 11m	St, MTX, CsA, NSAID, Cy, anti-TNF α , IVIG	GD, severe myelosuppression	12y, 4m	1y
21	B	F	Syst	2y, 11m	St, MTX, NSAID, IVIG	GD, cataracts, avascular necrosis of femoral head	11y, 1m	2y, 6m
22	B	M	Syst	8m	St, MTX, CsA, NSAID	Growth delay, cataracts, avascular necrosis of femoral head	8y, 9m	2y
23	B	F	Syst	4y, 3m	St, MTX, CsA, NSAID, Cy, IVIG	GD, Cushing, GI hemorrhage, avascular necrosis of femoral head	17y	3y, 6m
24	C	F	Syst	2y, 9m	St, MTX, CsA, NSAID, Cy, Chlor, IVIG, anti-TNF- α	GD, severe hypertension	5y	2y
25	C	F	Syst	9y, 2m	St, MTX, CsA, NSAID, Cy, Chlor, IVIG, anti-TNF α	GD, severe GI toxicity	14y, 9m	2y
26	C	F	Syst	2y, 8m	St, MTX, CsA, NSAID, Chlor, IVIG, anti-TNF α	GD, severe myelosuppression, hypertension, photosensitivity	9y, 9m	1y
27	B	M	Syst	10m	St, MTX, CsA, NSAID, Cy, anti-TNF α	GD, O, vertebral compression fracture	5y, 6m	1y, 6m
28	B	M	Syst	8y, 4m	St, MTX, CsA, NSAID	GD, O, avascular necrosis femoral head, vertebral compression fracture	11y	1y
29	B	F	Syst	7y, 1m	St, MTX, CsA, NSAID	GD, O, intestinal perforation, vertebral compression fracture	9y, 1m	†
30	A	F	Syst	2y, 1m	St, MTX, CsA, NSAID	GD, O, compression fractures	4y, 10m	2y, 6m
31	A	F	Syst	2y, 7m	St, MTX, CsA, NSAID, anti-TNF α	GD, O	8y, 5m	†
32	A	M	Syst	10y, 2m	St, MTX, CsA, NSAID, Cy, IVIG	GD, O, hepatic dysfunction	18y, 3m	3y
33	A	F	Syst	4y, 8m	St, CsA, MTX, NSAID	GD, O, severe hypertension	6y, 9m	1y, 6m
34	B	M	poly	3y, 3m	St, MTX, NSAID, IVIG	GD, O, vertebral fractures, cataracts	9y, 11m	1y, 6m
Total		19/15 (M/F)	29/5†	3y, 11m			9y, 5m	2y, 4m

anti-TNF α , anti-tumour necrosis factor α receptor therapy; ASCT, autologous stem cell transplantation; Auro = Auromyosine; Aza = Azathioprine; Chlor = Chlorambucil; CP, conditioning protocol; CsA, Ciclosporin A, Cy = Cyclophosphamide; F, female; FU, follow up (years and months); GD = growth delay; Hydroxy, hydroxychloroquine; IVIG, intravenous Immunoglobulins; m, months; M, male; MTX, methotrexate; NSAID, non-steroidal anti-inflammatory drug; O, osteoporosis; Plaq, plaquenil; poly, polyarticular JIA; St, steroids; Sulfa, sulfasalazine; Syst, systemic JIA; y, years.

† Death

† n systemic/n polyarticular

Statistical methods

Treatment efficacy was evaluated by testing whether there was a difference in the core set criteria scores between baseline and the three monthly evaluations after transplantation, using the Wilcoxon signed-rank test. The effectiveness of total body irradiation (TBI) was evaluated by comparison of core set criteria scores between patients treated with TBI and those treated without TBI, using a Mann-Whitney U test. In order to evaluate the extent and duration of immunosuppression, the Wilcoxon signed-rank test was used to assess whether there were significant differences between baseline and the three monthly measurements of laboratory variables. To test whether there was a difference in T-cell recovery between patients with a complete response and those with no or partial response, or between patients treated with different conditioning regimens, a Mann-Whitney U test was used. Correlations were calculated by linear regression.

Results

Clinical characteristics before ASCT

The clinical characteristics before ASCT in all children were a polyarticular course complicated by erosions, osteoporosis, and stunted growth (Table 1). In addition, all children with systemic JIA suffered periods of spiking fever, exanthemata and severe corticosteroid related side effects. Ten of the 34 children had failed treatment with anti-TNF α therapy. The mean time interval between diagnosis and transplant was 5 years, 4 months (standard error of mean (SEM), 6 months). More details are shown in Table 1.

Inclusion and exclusion criteria for ASCT

In order to be eligible for ASCT, each center used the agreed inclusion and exclusion criteria (18-19). The inclusion criteria were failure to respond to high dose methotrexate given by the intramuscular or subcutaneous routes (1 mg/kg/wk or 15 mg/m²), in addition to failure to respond to at least one other disease modifying anti-rheumatic drug (DMARD), corticosteroid dependency, or unacceptable toxicity from DMARDs or corticosteroids. After anti-TNF α treatment became available, failure to respond to these agents was added to the inclusion criteria. Exclusion criteria were cardiorespiratory insufficiency, chronic active infection, persistent fever, and other signs of systemic disease activity despite corticosteroids at time of assessment for transplant, end stage disease (Steinbrocker IV), or poor compliance.

Bone marrow harvest and T-cell depletion.

In 25 patients, bone marrow was harvested at least one month before ASCT. In nine patients autologous haematopoietic stem cells were mobilised into the peripheral blood using a single infusion of cyclophosphamide at 1.5-3.0 g/m² and granulocyte colony stimulating factor (G-CSF) at 10 μ g/kg/d. The graft was either purged by T- cell depletion with CD2 and CD3 antibodies or by positive stem cell selection using CD34 selection devices. In the nine cases of peripheral bone marrow

collection these techniques yielded a final suspension with a CD34+ cell count of $2.9\text{--}10.9 \times 10^6$ cells/kg (mean 5.2×10^6 cells/kg) and with a CD3+ cell count of $0\text{--}1.4 \times 10^5$ cells/kg (mean 0.3×10^5 cells/kg). In the 25 cases of bone marrow collection these techniques yielded a final suspension with a CD34+ cell count of $0.44\text{--}6.0 \times 10^6$ cells/kg (mean 2.2×10^6 cells/kg) and with a CD3+ cell count of $0\text{--}3.5 \times 10^5$ cells/kg (mean 0.7×10^5 cells/kg). The cell suspensions were frozen and stored until reinfusion (31).

Conditioning for ASCT.

In all, three different conditioning regimens were used (Table 2). Conditioning regimen A (23 patients) included four days of anti-thymocyte rabbit immunoglobulin (ATG, SangStat, Fremont, California, USA), 5 mg/kg from day -9 to -6, cyclophosphamide, 50 mg/kg/day from day -5 to -2 and low dose total body irradiation (TBI, 4 Gy, single fraction) on day -1. Conditioning regimen B (eight patients) included the above scheme without TBI. Conditioning regimen C (three patients) included fludarabine (30 mg/m^2) on day -7 and day -6, cyclophosphamide (50 mg/kg/day) from day -5 to day -2, ATG (5 mg/kg) from day -6 to day -3, methylprednisolone (1 g/m^2) from day -4 to -2 and no TBI. In all 34 children the frozen stem cell suspension was thawed and infused at day 0. Methotrexate and Cyclosporin A were stopped before ASCT and prednisone was tapered when possible, starting not earlier than 2 months after ASCT. Disease flare up or relapse was treated in the same way as before ASCT.

Engraftment and immunological reconstitution.

Engraftment occurred in all 34 patients with full haematological recovery. Detailed immunological reconstitution data were available for 29 patients (85%). In these 29 patients neutrophil recovery ($> 0.5 \times 10^9/\text{l}$) occurred between day 8 and day 30 post- ASCT (mean (SEM), 16 (1.1) days). Details of T-cell recovery are given in Fig. 1(32). All children suffered prolonged depression of CD4+ T cells, a common immunological feature also seen after intense chemotherapy (33,34). Twelve months after ASCT (range 6 to 18 months), the numbers of CD4+ T cells were normal compared with baseline (Wilcoxon signed-rank test). A relatively fast reconstitution of CD8+ T cells resulted in an inverted CD4+/CD8+ T-cell ratio in 24 of 29 patients (83%) lasting 3 to 18 months (mean (SEM), 9.2 (1.1) months). An early predominance of CD45RO+ memory cells was followed by the reappearance of CD45RA+ naive cells after six to nine months (Figure 1). Three to 18 months after ASCT (mean (SEM), 10.5 (1.6) months), in vitro mitogenic responses were normal. No differences were seen in T-cell recovery between patients with a complete response and patients with no or partial response, or between patients treated with different regimens.

General outcome.

Complete data on six core set criteria (baseline and at three months interval) were available for 22 of the 34 patients (65%). For the other 12 patients data on three to five core set criteria at three month intervals were available, always including the number of active joints. In these patients only three or more criteria were used to make an estimation of the improvement.

The general outcome of the patients is summarised in Table 2. Eighteen of the 34 patients (53%) with a follow up of 12 to 60 months (mean (SD); 29 (12) months) had complete drug-free remission. Seven of these patients had previously failed treatment with anti-TNF α therapy. Five patients, who later achieved complete drug-free remission, had flare up of disease at three to six months post-ASCT; these events occurred either spontaneously while tapering the prednisone or following infection. Slower tapering of prednisone or low dose NSAID, and in one case intra-articular corticosteroids, was enough to control the flare up.

No response was noted in seven of the 34 patients (21%). After an initial phase of improvement during immunoablation, these patients showed a complete relapse of the disease, three to 17 months after ASCT, which was as severe as before and needed treatment with high doses of prednisone, methotrexate, anti-TNF α therapy, cyclosporin A, and in one case even cyclophosphamide pulse therapy. In these patients the disease was as refractory to corticosteroids and other antirheumatic drugs as before ASCT. Two other patients who showed a complete relapse died 13 and 16 months after ASCT. The first patient developed a full-blown relapse 13 months after ASCT and was treated with high dose prednisone, methotrexate and cyclophosphamide pulse therapy. While waiting for HLA-matched allogenic bone marrow he developed an EBV infection and died of hepatic failure, being severely immunosuppressed. The second patient developed a primary varicella zoster virus (VZV) infection 13 months after transplantation. At that time she was being treated with anti-TNF α therapy (etanercept) and methotrexate. Antirheumatic drugs were stopped and the patient was treated successfully with aciclovir. However, two months later she unexpectedly developed jaundice and died of liver failure of unknown etiology.

Table 2. Clinical improvement after autologous stem cell transplantation at maximal follow up

Center	Protocol	Complete remission	Partial response			No response	TRM	Total
			30%	50%	70%			
Utrecht ¹	A	5	-	3	1	2*	1	12
Leiden ²	A	3	1	-	-	2	1	7
London ³	A	1	-	-	-	-	-	1
Gothenburg ⁴	A	1	1	-	-	1*	-	3
Brussels ⁵	B	3	-	-	-	-	-	3
Halle-Wittenberg ⁶	B	1	-	-	-	-	-	1
Paris ⁷	B	1	-	-	-	1	1	3
Newcastle ⁸	B	1	-	-	-	-	-	1
Jena ⁹	C	2	-	-	-	1	-	3
Total		18	2	3	1	7	3	34

* One case in Utrecht and one in Gothenburg of disease related causes¹

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TRM = Transplant Related Mortality

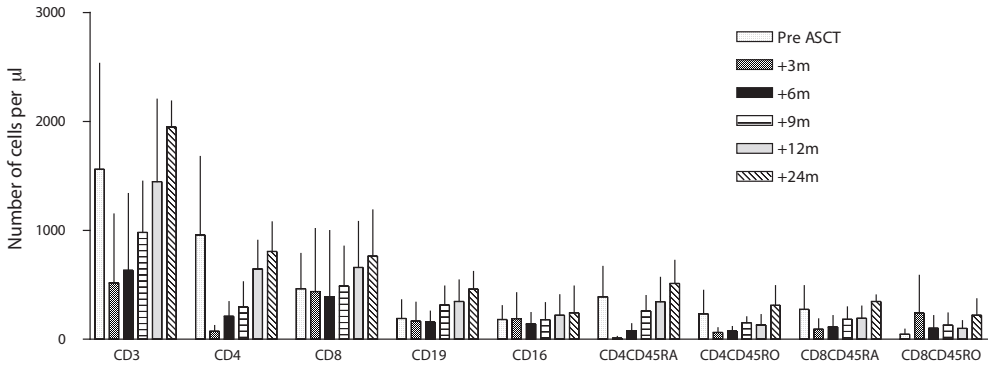


Figure 1. Reconstitution of lymphocyte subsets in 29 JIA patients after ASCT for 24 months. Error bars=SEM.

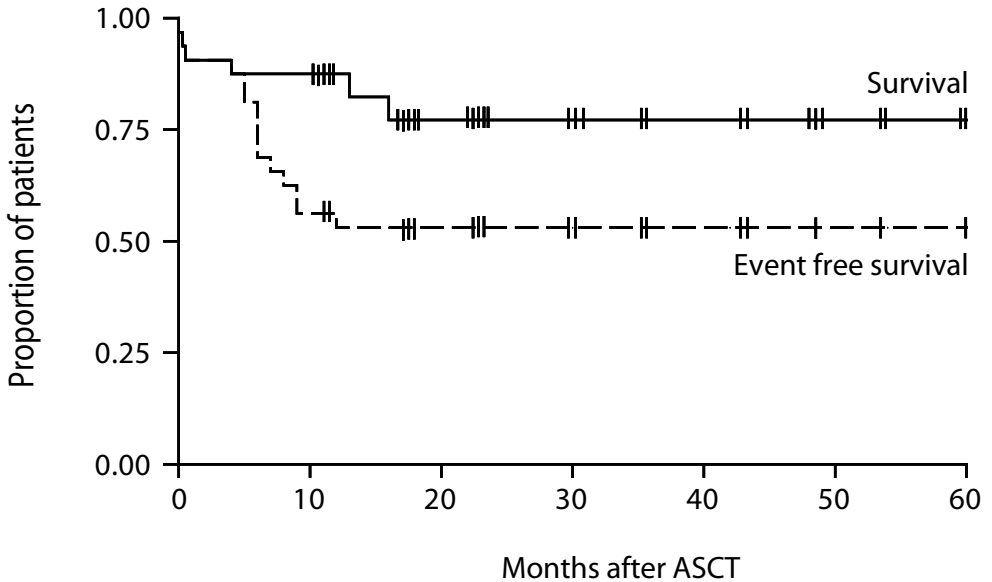


Figure 2. Kaplan Meier curves showing the proportion of surviving patients and the proportion of event-free surviving patients. An event is defined as either a partial or complete recurrence of disease. Each bar represents the maximal follow up of a particular patient.

Six of the 34 patients (18%) showed a partial response. One of these patients had previously failed anti-TNF α therapy. In these six patients, treatment with either low dose prednisone (two patients, 2.5 and 5 mg prednisone daily) or other antirheumatic drugs was reinstated at the time of the relapse, resulting in a subsequent improvement in the disease. Using the Giannini core set criteria for disease improvement (with modifications as above), these patients- who were refractory to these

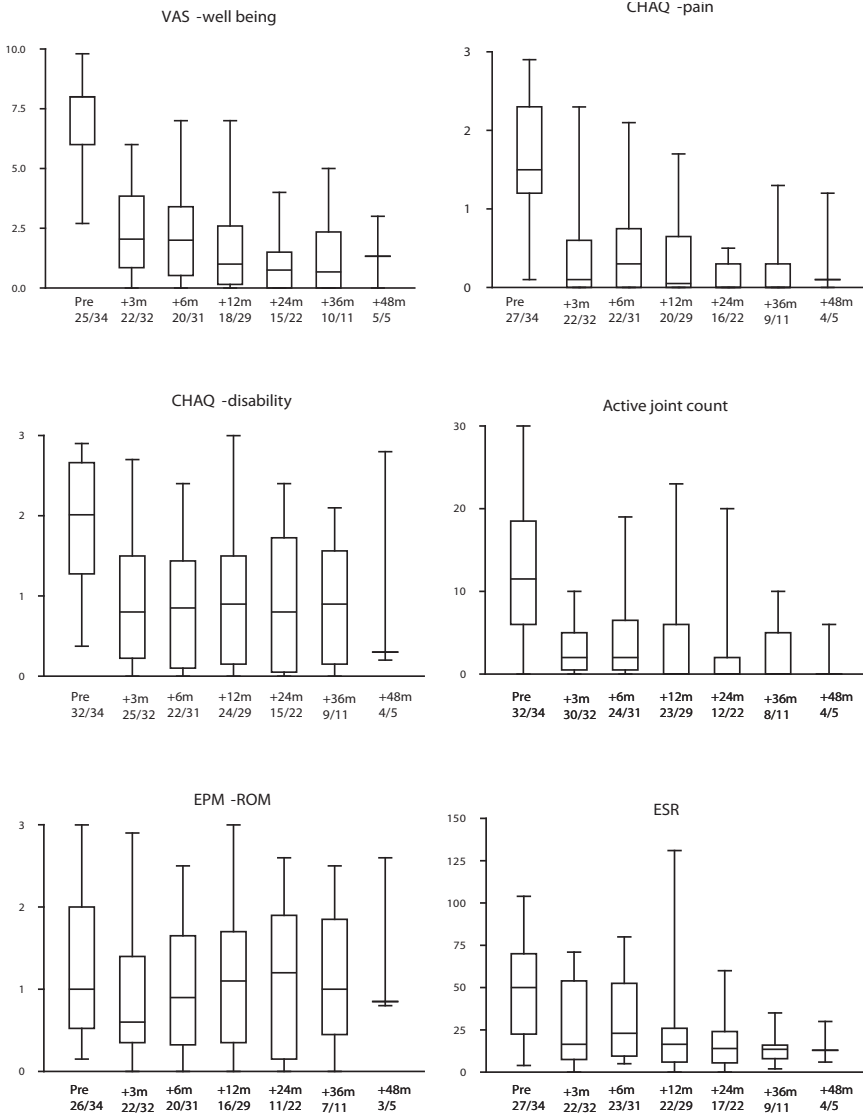


Figure 3. Rheumatological follow up before and at three monthly intervals after ASCT. Error bars=SD. Numbers below the x axis indicate ratios of the available data (number of data available/number of patients with indicated follow-up). With the exception of EPM-ROM scores at time points 24, 36 and 48 months post-ASCT, all scores post-ASCT were significantly decreased when compared to baseline (Wilcoxon signed-rank test, all p values \leq 0.04). Top left: physician’s global assessment of overall wellbeing (visual analogue scale, range 0-10); top right: parent/patient assessment of pain (VAS, range 0-3); middle left: functional disability (child health assessment questionnaire (CHAQ), range 0-3); middle right: number of active joints (0-54); bottom left: number of joints with limited range of motion (pediatric EPM-ROM, range 0-3); bottom right: erythrocyte sedimentation rate (ESR, mm/hour).

Table 3. Infectious complications after ASCT.

Case	Infection	Time*	Case	Infection	Time*
1	VZV, skin	<3m	18	HSV type 1, throat	< 3m
2	<i>Mycobacterium Szulgai</i> , eye	6m	19	<i>Streptococcus mitis</i> , blood	< 3m
				Generalized VZV	< 3m
				Primo CMV	< 3m
				Candida, oesophagitis	< 3m
3	<i>Xanthomonas maltophilia</i> , stool	<3m	20	None	
	HSV, hepatitis	24m			
4	EBV, disseminated †	12m	21	VZV, skin †	12m
	<i>Streptococcus viridans</i> , blood	<3m			
5	VZV, skin	<3m	22	None	
6	VZV, skin	6m	23	Cryptosporidium, stool	< 3m
	Hepatitis A	18m			
7	Streptococcus, blood	<3m	24	None	
	VZV, skin	18m			
8	CMV reactivation	4m	25	Candida oesophagitis	< 3m
	EBV (†, MAS)	4m			
	Candida oesophagitis	<3m			
9	CNS, blood	<3m	26	Toxoplasmosis, generalized (†, MAS)	< 3m
	VZV, skin	9m			
10	None		27	None	
11	<i>H. Influenzae</i> , blood	<3m	28	Parainfluenza, blood	< 3m
	CNS, blood	<3m		CMV reactivation, blood	< 3m
	VZV, skin	9m			
12	None		29	Legionella pneumonia	< 3m
				CMV reactivation	< 3m
13	VZV skin	<3m	30	CNS, blood	< 3m
	CMV reactivation	<3m		CMV reactivation, blood	< 3m
				HSV, stomatitis	< 3m
				Parainfluenza, blood	< 3m
14	none		31	CNS, blood	< 3m
15	CNS, blood	<3m	32	None	
	Adenovirus type 3, faeces †	<3m			
16	HSV type 1, throat	12m	33	None	
	Influenza A, nose	<3m			
	VZV, skin	4m			
17	<i>Streptococcus mitis</i> , blood	< 3m	34	↑ EBV genome copies	< 3m
	CMV reactivation	< 3m			
	VZV, skin	12m			

* Time point after autologous stem cell transplantation.

† Death.

CMV, cytomegalovirus; CNS, coagulase negative staphylococcus; EBV, Epstein-Barr virus; HSV, herpes simplex virus; MAS, macrophage activation syndrome; VZV, varicella-zoster virus.

drugs before ASCT- now showed a 30-70% improvement in their disease. Figure 2 shows Kaplan-Meier curves with the proportion of surviving patients and the proportion of surviving patients free of either a partial or complete recurrence of the disease. Interestingly, all cases of mortality and all partial and complete relapses of disease occurred in the first 18 months after ASCT.

Table 4. Currently used protocols and proposed new protocol of ASCT for JIA

Protocol	Conditioning	TCD	Follow-up (months)	Inclusion criteria	Exclusion criteria	Supportive care
A	Cy, ATG, TBI (low dose, 4 Gy)	CD2/3 or CD34	60	Drug resistant, including anti-TNF α	Cardio-respiratory insufficiency, chronic infections, active systemic disease at time of ASCT, end stage disease, poor compliance.	No specific rules for IVIG or antimicrobial prophylaxis
B	Cy, ATG	CD34	42			
C	Cy, Flu, ATG	VCR or CD34	24			
Proposed new protocol	Cy (50 mg/kg/d at day -4 and -1) ATG(5mg/kg/d at day -8 to day-5) Reinfusion of stem cells at day 0	CD34 selection CD34> 1x 10 ⁶ /kg and add back CD3 1x10 ⁶ /kg		Same as above	Same as above	Isolation during aplastic period. IVIG (0.4 mg/kg x 3 weeks) until CD3 count > 500/ μ L Pentamidine (PCP) prophylaxis, avoid cotrimoxazol

ASCT, autologous stem cell transplantation; ATG = anti-thymocyte rabbit immunoglobulin (SangStat, Fremont, California, USA) ; CD2/3, negative selection by monoclonal antibodies to CD2 or CD3 positive lymphocytes; CD34, positive selection of CD34+ stem cells; Cy, Cyclophosphamide; Flu, fludarabin; IVIG, intravenous immunoglobulin; PCP, *Pneumocystis Carinii* pneumonia; TBI, total body irradiation; TCD, T cell depletion using; anti-TNF α , anti tumour necrosis factor α ; VCR, *ex-vivo* T-cell depletion using vincristine.

Rheumatological follow up

Figure 3 shows the available rheumatological follow up data for all patients. With the exception of EPM-ROM scores at time points 24, 36 and 48 months post-ASCT, there was a significant decrease in all core set criteria scores at time points 3, 6, 12, 24, 36 and 48 months after ASCT ($p \leq 0.04$). No correlation could be found between stem cell source, number of reinfused T cells, or engraftment and the clinical outcome. No statistically significant difference was seen in core set criteria scores obtained 12 and 24 months after ASCT between patients conditioned with protocol A (with TBI) and protocol B or C (no TBI).

Transplant related complications and mortality

All children developed chills, fever and malaise during infusion of ATG. After ASCT, 24 of the 34 children (71%) developed at least one infection, mainly during the aplastic period (Table 3). During the aplastic period six children developed septicaemia; four blood cultures were positive for coagulase negative staphylococci and two for *Streptococcus mitis*. These cases all responded well to intravenous antibiotics. Ten patients developed a limited and one a generalized varicella-zoster virus eruption, three to 18 months after ASCT, successfully treated with aciclovir. One patient had a primary cytomegalovirus (CMV) infection and six had CMV reactivation one to 12 months post-ASCT. One case each of localised atypical mycobacterial infection (six months after ASCT), *Legionella pneumoniae* infection (three months after ASCT), hepatitis A infection (18 months after ASCT), and herpes simplex virus (HSV) hepatitis (24 months after ASCT) were seen; all resolved completely.

Three patients with systemic JIA died shortly after ASCT of infectious complications. The first died of an infection associated haemophagocytic syndrome (IAHS), also called macrophage activation syndrome (MAS), induced by Epstein-Barr virus (EBV) four months after ASCT. At the time of the EBV infection, her JIA was in remission. The second fatal IAHS occurred 18 days after transplant, while the patient was still in aplasia. A third fatality, resembling IAHS, occurred 10 days after ASCT and was caused by a disseminated toxoplasmosis infection (35,36). Two further patients died 13 and 16 months after ASCT as described above.

Discussion

Our study indicates that ASCT in severely ill patients with JIA induces complete drug-free remission in a substantial proportion of the patients (18 of 34 patients, 53%), even after prolonged withdrawal of anti-rheumatic drugs. Also the patients who achieved a partial response (six of 34, 18%) showed a remarkable improvement in most core set criteria, indicating a profound increase in general wellbeing. The limited improvement in paediatric EPM-ROM and CHAQ-disability scores after ASCT shows that the erosive joint destruction which existed before ASCT was not reversed during the post-ASCT follow up in this study and might well turn out to be irreversible over a longer follow up. Yearly x-rays of the joints in a substantial proportion of the children who went into complete remission after ASCT (data not shown) suggested, however, that the progression of destruction had arrested after transplantation.

The high mortality in this study raises the issue of benefit versus risk of this procedure. Though mortality is seldom an immediate issue in most patients with JIA, various series published between 1981 and 1991 report a mortality between 8% and 14% in children with systemic onset JIA (10% of all cases) (37-41). There is no doubt that with better treatment strategies the mortality has decreased in the last decade, but it is still estimated to be more than 2% (6). The causes of death are quite evenly divided between disease related causes and treatment related causes. The high overall mortality in the group of children treated with ASCT therefore partly reflects the selection of most severely ill patients. Infection associated haemophagocytic syndrome (IAHS) caused three of the five deaths in the present study. There is circumstantial evidence for a relation between systemic JIA and IAHS (42, 43). IAHS and systemic JIA share symptoms such as spiking fever, and untreated systemic JIA can progress to IAHS, with a high mortality (44, 45). The occurrence of IAHS in systemic JIA after bone marrow transplantation may be caused by stringent T cell depletion, resulting in inadequate control of macrophage activation (42). In 1999 an international agreement was achieved on adaptation of the protocol, consisting of a less profound T-cell depletion, control of systemic disease before transplantation, and a slow tapering of corticosteroids after ASCT (46). After adaptation of the protocol no further cases of IAHS have occurred.

The high frequency of infection in this study, including two deaths, illustrates the vulnerability of the patient group after ASCT. Whether the two patients dying of infection at 13 and 15 months after ASCT need to be considered as cases of transplant related or disease related mortality is questionable.

Both patients were severely immunosuppressed as a result of high dose immunosuppressive drugs which were restarted because of a full blown relapse. Furthermore, though the immune system had fully reconstituted, it has been shown that after autografting severe immunodeficiency associated with persisting oligoclonality may continue for several years (33,47,48). Awareness that both factors amplify the immune suppression is important. Early recognition with immediate treatment of infections in this group of patients is even more important than before ASCT. Future protocols therefore need to include the prophylactic administration of antiviral drugs and intravenous immunoglobulines until normal CD4+ T-cell counts have returned, with frequent EBV monitoring by polymerase chain reaction. We believe that with the adaptations made to the protocol in 1999 to reduce MAS, the administration of prophylactics after ASCT, and an awareness of the risks of restarting immunosuppressive drugs shortly after ASCT, mortality can be reduced significantly.

Since the availability of anti-TNF α therapy, failure of response to this treatment has been added as an inclusion criterion for future cases. The effects of etanercept in polyarticular JIA are impressive, with a response rate up to 80% of methotrexate resistant patients with polyarticular JIA (9). So far no formal study has been undertaken on children with systemic JIA, but first experiences with anti-TNF α therapy in patients with persistent systemic features have shown several treatment failures (11,12). Furthermore, the potential for severe adverse events with anti-TNF α agents serves as an unfortunate limitation to this treatment (49-51). Among the 34 children in this study were eight with systemic JIA who had previously failed treatment with anti-TNF α therapy; all eight responded extremely well to ASCT, confirming the possible role of this procedure in patients with severe disease.

The mechanism underlying the occurrence of complete remission after ASCT seems obvious. The rationale of the treatment is eradication of the autoreactive lymphocytes and memory cells by a lymphoablative conditioning regimen, and subsequent rescue with T-cell depleted autologous bone marrow. Regeneration of the T-cell population occurs in the thymus by a process of proliferation and tolerisation to self, resulting in a "reset" immune system. Though it is generally thought that the recurrence of disease post-ASCT either reflects pathogenic cells in the stem cell graft or those remaining in the host, evidence is mounting that further depletion of T cells is not the answer to improving the outcome. In our study no correlation could be found between the number of reinfused T cells and the clinical outcome. This finding is consistent with a recent study by Moore *et al.* showing similar outcomes in severe rheumatoid patients undergoing haemopoietic stem cell transplantation with unmanipulated cells and those receiving CD34 selected cells (52). Furthermore, five patients in our study showed a benign, transient, oligoarticular synovitis before achieving complete remission, suggesting that some autoimmune T cells are still present after ASCT. The self limiting character of the synovitis favours the concept that ASCT not only resets autoimmune T cells, but also restores the regulation of these cells (53).

The above issue raises the question whether T-cell depletion of the marrow is crucial to the process of the transplant, or whether an intense but non-myeloablative regimen (with or without stem cell support) would be just as effective. Thus far, the conditioning agents used have been selected to maximise T-cell eradication. Comparison of various conditioning regimens in animal models indicates that a combination conditioning with high dose cyclophosphamide and low dose TBI is optimal

(54). The use of TBI in children remains, however, controversial because of concerns about long term safety. Though the limited number of patients does not allow us to draw definitive conclusions, our present study suggests that children not given TBI have equally good outcome as those treated with irradiation. Given the obvious concerns over the use of TBI we propose to eliminate TBI from future conditioning regimens. Table 4 shows a proposed new protocol. A good controlled, prospective study is now needed to investigate the effectiveness of the new protocol.

Conclusions

Autologous stem cell transplantation in severely ill patients with JIA induces drug-free remission of the disease and profound improvement in general wellbeing in a substantial proportion of patients. However, the procedure carries a significant mortality risk, making it necessary to weigh carefully the morbidity and mortality risks of the prolonged immunosuppression of “conventional” treatment against those of the short but intense immunosuppression of ASCT.

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