High dose chemotherapy and autologous hematopoietic stem cell transplantation for rheumatoid arthritis

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CHAPTER 4

HIGH DOSE CHEMOTHERAPY AND AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION IN PATIENTS WITH RHEUMATOID ARTHRITIS.

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Abstract.
Objective. To assess the feasibility, safety and efficacy of high dose chemotherapy and autologous hematopoietic stem cell transplantation (HSCT) in patients with severe, refractory rheumatoid arthritis (RA).
Methods. Fourteen patients (3 male, 11 female, mean age 43 yrs, mean disease duration 10 yrs) with active, destructive, refractory RA entered the study. Autologous hematopoietic stem cells were collected by leukapheresis after mobilization with a single infusion of cyclophosphamide (4 gr/m^2) and s.c. injections of filgrastim (G-CSF). Immunomagnetic selection of $CD34^+$ cells from the leukapheresis products was performed to deplete potentially autoreactive lymphocytes. The conditioning regimen consisted of intravenous administration of high doses of cyclophosphamide (cumulative dose 200 mg/kg), with subsequent reinfusion of the graft. Patients were monitored for disease activity, disability, adverse effects, and hematopoietic and immunologic reconstitution.
Results. All 14 patients completed the mobilization and leukapheresis procedures successfully, 12 proceeded to conditioning and transplantation. Engraftment occurred in all of these patients, with rapid hematologic recovery. No major unexpected toxicity was observed. Marked improvement of disease activity was recorded in 8/12 patients at more than 50% of the visits, with a follow-up ranging from 7-21 months. The clinical responders included 2 patients who previously failed TNF-blocking agents.
Conclusions. High dose chemotherapy followed by autologous hematopoietic stem cell transplantation is feasible and safe, and can result in long term improvement of disease activity in patients who previously failed conventional antirheumatic drugs and TNF-blocking agents. The persistence of active disease in some patients may reflect heterogeneity of the underlying disease process.

Introduction.

A new treatment approach, involving intense immunosuppression and autologous hematopoietic stem cell transplantation (HSCT), has emerged in recent years for the treatment of severe, refractory rheumatic autoimmune diseases including rheumatoid arthritis (RA) [1-4]. The rationale of this strategy is based on the concept of immunoablation by intense immunosuppression with subsequent regeneration of naive T lymphocytes derived from reinfused hematopoietic progenitor cells [5]. In several patients with intractable RA, long term remissions were observed with this strategy, although failures have been reported as well [6,7]. Only small numbers of RA patients have been treated thus far. Although different treatment protocols have been used, high dose chemotherapy (HDC) as a means to achieve immunoablation has been invariably used in all studies. To extend previous findings on selected cases, we conducted an open study to investigate the feasibility, safety, and efficacy of HDC + HSCT in a cohort of patients with therapy-refractory, active, destructive RA. In addition, the extent and duration of immunoablation was assessed, and the relationship between immunological changes and the clinical responses resulting from a period of intense immunosuppression was examined.

Patients and methods.

Patient selection. This was a multicenter, open-label phase I/II study. The protocol was approved by the ethics committees from the participating institutions. All patients provided written informed consent. Eligibility criteria were as follows: a. established diagnosis of rheumatoid arthritis according to ACR-criteria [8], b. progressively erosive disease with large joint involvement, c. failure to respond to ≥ 4 second-line drugs including maximal tolerable dose of methotrexate and combination therapy, d. active disease as defined by ≥ 6 swollen joints and ≥ 6 tender joints and ≥ 1 hour of morning stiffness, and e. age 18-60 years. Exclusion criteria were: a. pulmonary impairment, defined as total lung capacity, vital lung capacity or diffusion capacity < 70% of predicted values, b. cardiac impairment, defined as clinical evidence of heart failure with a left ventricular ejection fraction of < 50%, c. liver disease, defined as ASAT or ALAT or bilirubin > 2x upper limit of normal on 2 repeated tests, d. renal impairment, defined as creatinine clearance < 70 ml/min, e. white blood cell count < 2.0 $x10^9$/l, platelet count < 100 $x10^9$/l, hemoglobin < 6.0 mmol/l, f. acute or chronic infection, g. positive test for HIV, h. concurrent neoplastic disease or evidence of myelodysplasia, i. uncontrolled systemic hypertension, j. active peptic ulcer disease, k. positive pregnancy test, l. previous joint arthroplasty and m. concomitant therapy with anticoagulant drugs.

Treatment schedule. Autologous hematopoietic stem cells were mobilized using a single infusion of cyclophosphamide (CyC) 4 g/m^2 followed by filgrastim (G-CSF) 10 μg/kg/day subcutaneously until leukapheresis. Administration of filgrastim commenced 5 days after the CyC infusion. Patients underwent leukapheresis as soon as the $CD34^+$ blood levels exceeded 20 x 10^3/ml. Leukapheresis was performed on a continuous flow cell separator machine to obtain at least 5 x 10^6 $CD34^+$ cells / kg body weight. Immunomagnetic selection of $CD34^+$ cells from the leukapheresis product was performed using the Clinimacs Device (Miltenyi Biotec, Munich, Germany), aimed at obtaining a minimum of 2 x 10^6 $CD34^+$ cells/kg and a maximum of 2 x 10^4 $CD3^+$ cells/kg. All disease-modifying antirheumatic drugs (DMARDs) were discontinued before mobilization and corticosteroids were tapered thereafter when possible. NSAIDs were continued in the lowest dosage needed to control pain and morning stiffness. The conditioning regimen consisted of CyC 50 mg/kg/day intravenously for 4 consecutive days (total 200 mg/kg). Hyperhydration, alkalinisation of urine and mesna were given in order to prevent hemorrhagic cystitis. The interval between the last dose of CyC and infusion of the stem cells was at least 48 hours. Following transplantation, patients were nursed in laminar flow rooms. All blood products were irradiated prior to infusion (25 Gy). Antibiotic decontamination and anti-emetic treatment were given according to local practice. The use of corticosteroids as anti-emetic therapy was left to the institution's practice. Patients treated in LUMC [8] all received methylprednisolone at a dose of 2 mg/kg during

conditioning for six consecutive days. In the other institutions (UMCU, UMCN, 4 patients) no steroids were given.

Assessment of toxicity. Safety was assessed according to WHO toxicity criteria. Furthermore, the units of transfused red blood cells and units of transfused platelets, infections, number of days of hospitalization and rehospitalization records were recorded as well.

Assessment of efficacy. The following clinical and laboratory investigations were performed at screening, prior to stem cell mobilisation (considered baseline), before conditioning and every three months after transplantation: physical examination including swollen joint count (SJC; 0-66), tender joint count (TJC; 0-68) and the Ritchie articular index (0-78), health assessment questionnaire (HAQ; 0-3), patient pain visual analogue scale (VAS; 0-10), patient disease activity VAS (0-10), physician global assessment of disease activity (0-10).

Laboratory measurements were performed on the same time points and included ESR, Hb, Ht, WBC with differential, platelet count, C-reactive protein (CRP), IgM rheumatoid factor, anti-cyclic citrullinated peptide (anti-CCP), total serum IgM, IgG and IgA.

Based on the above mentioned data efficacy was determined by the four variable disease activity score (DAS) (primary study parameter) [9], the ACR response criteria [10], and the health assessment questionnaire (HAQ).

Flow cytometric detection of cell surface antigens. Immunophenotyping studies were done on peripheral blood mononuclear cells obtained at baseline (prior to stem cell mobilisation), prior to conditioning and at 3, 6, 9 and 12 months after transplantation. The following combination of markers were used in order to identify different cell types: CD45-FITC (Becton Dickinson, San Jose, USA (BD))/CD14-PE (Dako, Glostrup, Denmark), CD3-FITC (BD)/CD4-PE (BD), CD3-FITC (BD)/CD8-PE (BD), CD3-FITC (BD)/CD16+CD56-PE (BD), HLA-DR-FITC (Dako)/CD3-PE (BD), CD10-FITC (DAKO)/CD20-PE (BD), CD19-FITC (BD)/CD5-PE (DAKO), CD45RO-FITC (DAKO)/CD4-PE (BD), CD45RA-FITC (BD)/ CD4-PE (BD), CD45RO-FITC (DAKO)/ CD8-PE (BD), CD45RA-FITC (BD)/ CD8-PE (BD).

Statistical analysis. Treatment efficacy was evaluated by testing whether there was a difference in DAS and HAQ between baseline and 3 monthly evaluations after transplantation using the Wilcoxon signed-rank test. Responders were defined as those patients attaining a good response based on the EULAR response criteria for the DAS at 3 months after transplantation. The Wilcoxon rank-sum (Mann-Whitney U) test was used to determine whether the clinical response was consistent by testing the differences in HAQ and ACR-response categories between responders and non-responders. In order to evaluate the extent and duration of immunoablation, the Wilcoxon signed-rank test was used to assess whether there were significant differences between baseline and 3-monthly measurements of laboratory parameters. Furthermore, the relationship between the clinical response and immunological changes was evaluated by testing whether laboratory

parameters differed between responders and non-responders. Correlations were calculated by linear regression.

Results.

Patient data. Fourteen patients with active, progressively erosive, refractory RA entered the study (mean age 43 years, range 22-55, disease duration 10 years, range 2-20). All patients had received the maximal tolerable dose of methotrexate, 5 patients had also failed TNF-blockade (Table 1). All 14 patients completed the mobilization procedure successfully, 12 patients proceeded to conditioning and transplantation. One patient chose not to proceed to conditioning because of marked improvement of disease activity after mobilization, another patient was withdrawn from the study when pulmonary embolism was diagnosed before conditioning. Both median and mean intervals between the first dose cyclophosphamide for mobilization and reinfusion of stem cells was 56 days (range 32-90days). Baseline parameters of disease activity are summarized in table 2.
All patients had a disease activity score (DAS) > 3.7 at baseline, defined as high disease activity [11].

Graft. Immunomagnetic selection of $CD34^+$ cells from the leukapheresis products was performed to deplete potentially autoreactive cells using the Clinimacs Device (Miltenyi Biotec, Munich, Germany). After selection the grafts contained a median of 4.62×10^8 $CD34^+$ cells (range 2.77-7.45), corresponding to a median of 6.9×10^6 $CD34^+$ cells/kg bodyweight (range 4.8-11.1). The median number of $CD3^+$ T cells in the graft was $< 44.0 \times 10^4$ (range 8-100), corresponding to 3.74 log depletion (range 3.0-4.6). The median percentage of $CD3^+$ T cells in the infused product was < 0.1 (range 0.03-0.36%).

Toxicity. Nausea, vomiting and alopecia was observed in all patients. Other treatment related morbidities occurred in 9/12 patients and included thrombosis of the vena subclavia due to an i.v. catheter (1/12), hydradenitis (1/12), metrorrhagia (1/12), herpes zoster (2/12), pseudomembranous enterocolitis (1/12), pneumothorax (1/12) and febrile neutropenia necessitating temporary antibiotic treatment (7/12). In 2/7 of these patients a causative micro-organism was isolated. Total days of hospitalization (including mobilization) was 33 (range 25-53). Median number of hospitalization days due to fever was 5 (range 5-20) in 8 patients. Three patients experienced WHO grade 3 toxicity: 2 with intolerable diarrhea requiring therapy and 1 with elevations of bilirubin and ASAT, ALAT levels. Mortality did not occur.

Patient	Sex	Age	Disease duration	Rheumatoid factor	HLA DRB1 typing	Previous therapy
TX01	F	43	11	+	0301 / 0301	HCQ, gold i.m, SSZ, MTX, AZA, D-Pen, prednisone, CSA, CSA + MTX.
TX02	F	51	7	+	0404 / 1104	HCQ, gold oral, D-pen, MTX, AZA, SSZ, prednisone, MTX + prednisone.
TX03	F	52	15	+	0404 / 0801	HCQ, CSA, D-pen, gold i.m, SSZ, MTX, AZA, HCQ + AZA + prednisone; HCQ + CSA.
TX04	F	52	20	+	0301 / 0408	HCQ, gold i.m, D-pen, prednisone, MTX, SSZ, anti TNFα MTX + prednisone.
TX05	F	40	17	-	0101 / 0402	HCQ, SSZ, gold i.m, MTX, anti TNF, AZA, CSA, MTX + SSZ + HCQ.
TX06	F	55	12	+	0404 / 1101	HCQ, SSZ and MTX̃
TX07	F	35	9	+	0401 / 1201	HCQ, SSZ, prednisone, gold i.m, MTX, AZA, CSA, D-Pen, prednisone + MTX + SSZ + HCQ, Anti TNFα.
TX08	F	32	14	-	01 / 0301	SSZ, prednisone + MTX + gold i.m.
TX09	M	53	11	+	0404 / 1501	HCQ, gold, MTX, anti TNFα. MTX + CSA.
TX10	M	52	4	+	0101 / 0701	SSZ, prednisone + MTX, AZA + prednisone.
TX11	F	26	2	+	DR4 *	SSZ, MTX, gold i.m, AZA, anti TNF, prednisone.
TX12	F	48	8	+	DR1/DR15 *	Oral gold, D-pen, MTX, il-10, prednisone + HCQ.
TX13	F	22	9	+	DR14/DR17 *	SSZ, Oral gold, HCQ, MTX, CSA, CSA + MTX
TX14	M	38	5	+	DR1/DR11 5 *	MTX and MTX + HCQ.

Table 1. Patient characteristics. F = female, M = Male, MTX = methotrexate, HCQ = hydroxychloroquine, SSZ = Sulfasalazine, AZA = Azathioprine, D-Pen = D-penicillamine, CSA = Cyclosporin A, Anti TNF = TNF blocking agents. * Serologically determined.

Variable	Mean	Range
Swollen Joint Count (0-66)	24	7-39
Tender Joint Count (0-68)	25	11-49
VAS pain	6.6	2.2-9.7
VAS disease activity	6.2	2.0-8.4
HAQ	1.80	1-2.5
ESR	56	12-100
CRP	59	6-129
DAS	5.39	3.82-7.24

Table 2. Baseline characteristics of 14 study patients. VAS = visual analog scale; HAQ = Health Assessment Questionnaire; ESR = erythrocyte sedimentation rate; CRP = C-reactive protein; DAS = Disease Activity Score.

Engraftment. Engraftment occurred in all patients with rapid hematologic recovery. The median duration of neutropenia (defined as < 0.5 x 10^9 neutrophils/l) was 12 days (range 8 - 17 days), and median duration of platelet count < 20 x 10^9/l was 3 days (range 0-5) respectively. The average number of units of transfused red blood cells including transfusions after mobilization was 5.3 (range 0-9). The average number of units of platelet transfusions was 2.7 (range 0-5). The duration of neutropenia correlated with the age of the patient ($r = 0.65$, $p = 0.023$). The mean amount of cyclophosphamide used (including mobilization) was: 19,896 mg, median: 19,350 mg (range: 15,520 mg – 25,200 mg).
Clinical efficacy. Figure 1 shows the course of the mean DAS of 12 patients.
There was a significant decrease in DAS after HDC + HSCT at 3 ($p = 0.005$), 6 ($p = 0.003$) and 12 ($p = 0.018$) months. At 3 months post-transplantation 6 patients fulfilled the criteria for good response based on the EULAR response criteria for the DAS [11]. The other patients subsequently had either no (5) or a moderate response (1). Patients were divided in responders (good response) and non-responders (moderate and no response). The former group of patients ($n = 6$) all had a DAS < 2.4 (low disease activity), the latter group ($n = 6$) all had DAS > 3.7 (high disease activity) at 3 months after transplantation (Figure 2).

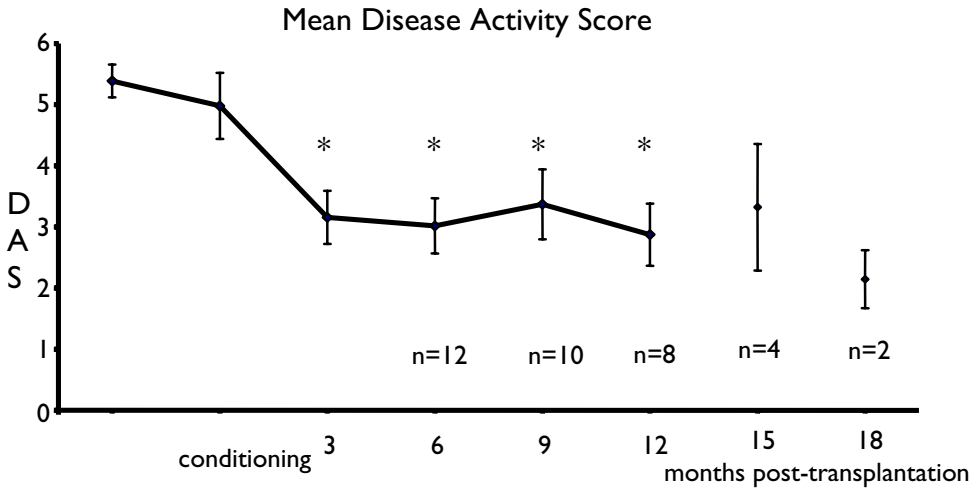

Figure 1. Mean DAS. DAS = (0.54 x √ Ritchie articular index) + (0.065 x number of swollen joints) + (0.33 x Ln ESR) + (0.0072 x patient disease activity VAS). * Significant change from baseline ($p < 0.01$). The error bars represent the standard error of the mean.

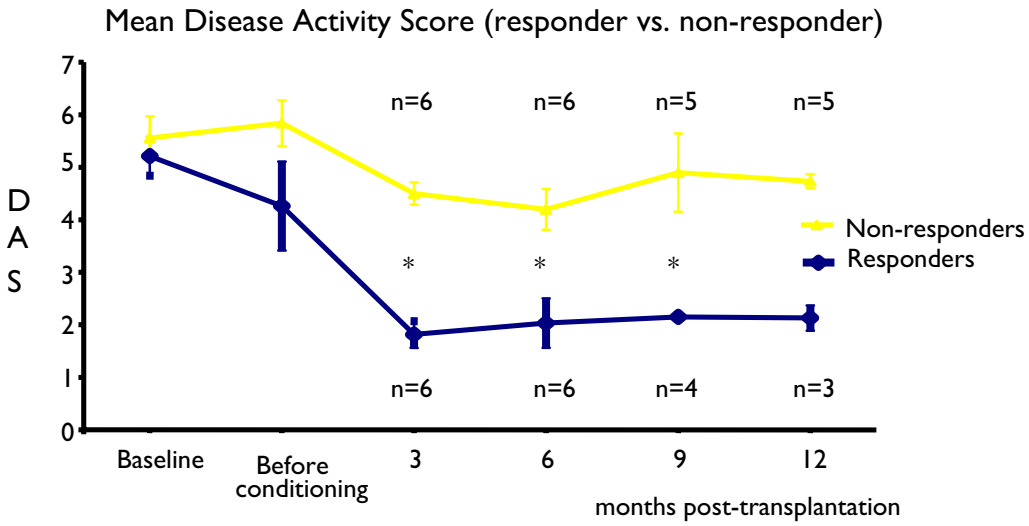

Figure 2. Mean DAS of responders and non-responders. Responders had DAS < 2.4 and ACR > 50% response and non-responders had DAS > 3.7 and ACR < 50% response at 3 months. The error bars represent the standard error of the mean. * Significant difference between responders and non-responders ($p < 0.05$).

There was a clear difference in DAS between patients who had a subsequent favorable disease course and those who did not. The difference in DAS between the two groups was statistically significant at 3 ($p = 0.004$), 6 ($p = 0.029$), 9 months ($p = 0.014$) . There was no statistical significance between responders and non-responders in DAS after mobilization ($p = 0.144$). At the 3 month evaluation no patient was on DMARDs. These results were similar when the ACR criteria for response were taken to determine clinical efficacy (responders satisfied the ACR 50% response criteria at 3 and at consecutive months of follow-up). Clinical efficacy according to the 20%, 50%, 70% ACR response criteria were at 3 months 8/12, 6/12, 3/12, at 6 months 8/12, 7/12, 2/12, at 12 months 5/8, 5/8 and 2/8 and at 15 months 3/4, 3/4, 2/4 (Table 3).

	3 months	6 months	12 months	15 months
70 %	3	2	2	2
50 %	6	7	5	3
20 %	8	8	5	3
No response	4	4	3	1

Table 3. Patients fulfilling the American College of Rheumatology criteria for improvement at 3, 6, 12 and 15 months of followup. Values are the no of patients.

Two patients fulfilled the DAS-criterion for remission (DAS < 1.6) [12] at 3 months, 3 patients at 6 months and 1 at 12 months. Responders included 2 patients who had failed TNF-blockade. In 7/12 patients DMARDs were reinstituted (minimally three months after transplantation) because of signs of active disease, resulting in amelioration of disease activity in 3/7 (2 patients received leflunomide, 1 patient methotrexate). The mean duration of DMARD-free period after transplantation was 130 days, median 105 days (range 99-204). Of the 7 patients who received DMARDs, one patient was a responder at t=3 months, 6 patients were not. The responder experienced an early relapse of disease activity one month after transplantation (DAS 7.41), which spontanously declined to 2.38. However signs of disease activity were still apparent. Upon reinstitution of MTX DAS decreased to 1.82. Of the 6 non-responders 2 responded favorably on reinstitution of DMARDs: from 4.83 to 2.82 and from 5.10 to 2.64 respectively. The 4 patients who failed to respond upon reinstitution to DMARDs had DAS ranging from 4.42 to 6.35 at 6 to 12 months after transplantation which was at least 3 months after the start of DMARDs. The follow-up of the mean HAQ showed statistically significant differences from baseline at 6 ($p = 0.005$) and 12 months ($p = 0.022$) (Figure 3). Figure 4 shows the mean HAQ of responders and non-responders. A statistically significant difference was found at 3 months ($p = 0.005$).

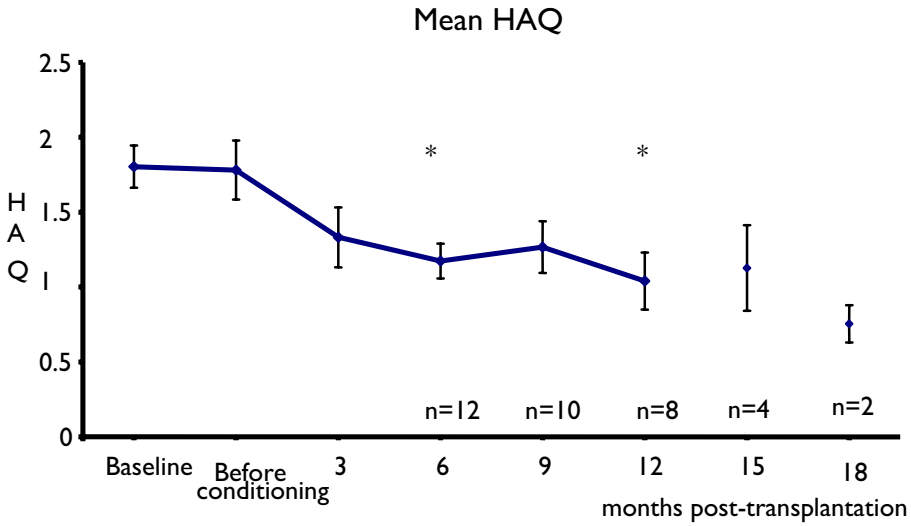

Figure 3. Mean health assessment questionnaire (HAQ) 0-3. * Significant change from baseline ($p < 0.05$). Bars show the mean ± SEM.

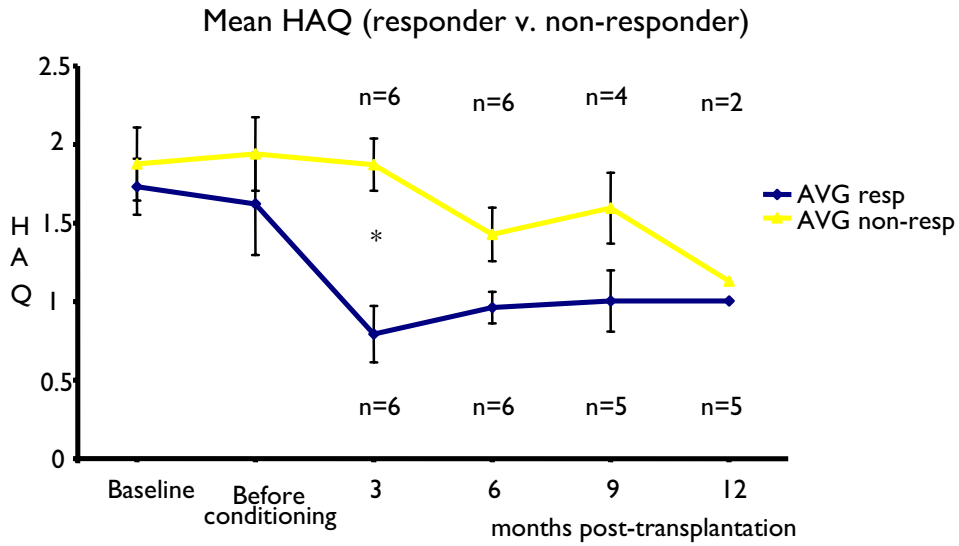

Figure 4. Mean HAQ of responders (AVG resp) and non-responders (AVG non-resp). Responders had DAS < 2.4 and ACR > 50% response and nonresponders had DAS > 3.7 and ACR < 50% response at 3 months. Bars show the mean ± SEM.
* Significant difference between responders and non-responders ($p < 0.05$). See figure 1-3 for definitions.

Laboratory measurements. Differences between baseline and 3-monthly measurements of laboratory parameters were assessed. An overall decrease in titre of IgM rheumatoid factor transplantation was observed, which was statistically significant at 3 ($p = 0.041$), 6 ($p = 0.011$) and 9 months ($p = 0.046$). The same was found for the anti-CCP, an antibody directed toward citrillunated peptides and specific for patients with RA [13] at 3 and 6 months (0.028 and 0.041). The relationship between clinical response and immunological changes was evaluated by testing whether laboratory parameters differed between responders and non-responders. No correlation was found in either IgM rheumatoid factor and anti-CCP levels between responders and non-responders at baseline or after transplantation. However, patients with a good clinical response had a significantly higher total IgG in peripheral blood than non-responders at baseline (mean 15.0 g/l vs. 9.03 g/l, $p = 0.004$) and after 3 months (mean 11.39 g/l vs. 7.85 g/l, $p = 0.025$). Furthermore there was a significant decrease in total IgG in the responder group after 3 months (mean 15.0 g/l vs. 11.39 g/l, $p = 0.028$).

Flow-cytometry. Immunophenotyping of peripheral blood mononuclear cells showed prolonged (> 6 months) depletion of $CD45RA^+$ T-cells after transplantation, whereas levels of $CD8^+$ cells, $CD19^+$ cells, $CD14^+$ cells and $CD3^-CD16^+/CD56^+$ cells quickly recovered. Levels of circulating CD8 ($p=0.5$), CD19 ($p=0.69$), CD14 ($p=0.14$) and CD3-CD16+CD56+ ($p=0.225$) cells at 1 year were not statistically significant different from baseline levels. The decrease in levels of circulating naive $CD4^+$ cells ($CD4^+CD45RA^+$) and memory $CD4^+$ cells ($CD4^+CD45RO^+$) was statistically significant at 3, 6 en 12 months ($p \leq 0.028$) (Figure 5.).

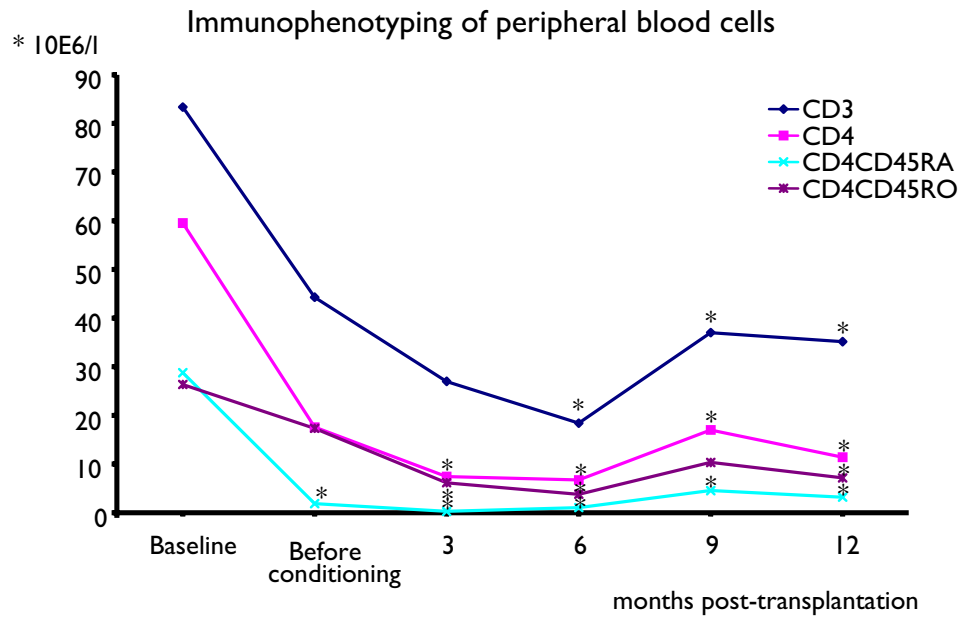

Figure 5. Immunophenotyping of peripheral blood mononuclear cells. Peripheral blood mononuclear cells were isolated by density gradient centrifugation on a Ficoll separation medium. Numbers of cells were calculated from the lymphocyte count and percentage of each subset determined by flow-cytometry. * Significant change from baseline ($p < 0.05$). *10E6/l = 10^6/liter.

Discussion.

Fourteen patients with intractable rheumatoid arthritis (RA) enrolled in an open phase I/II study on the clinical and immunological effects of high dose chemotherapy (HDC) and autologous hematopoietic stem cell transplantation (HSCT). Twelve patients completed the consecutive treatment steps: 1. mobilization of autologous hematopoietic stem cells with a single intravenous dose cyclophosphamide (4 g/m^2) and subsequent subcutaneous G-CSF injections, 2. leukapheresis with ex-vivo manipulation of the graft, 3. conditioning with cyclophosphamide (200 mg/kg), 4. autologous hematopoietic stem cell transplantation. One patient chose not to proceed to conditioning because of marked improvement of disease activity after mobilization. In another patient pulmonary embolism was diagnosed when he was admitted to undergo conditioning. Because of the absence of a temporal relationship, it was thought to be unrelated to the treatment protocol.

The treatment protocol was designed to combine practicality, safety and efficacy. For these reasons, we opted for a treatment regimen based on a single chemotherapeutic agent (cyclophosphamide), aiming at lymphoablation in stead of myeloablation, and CD34-selection of the graft to diminish the putative risk of reinfusing autoreactive or pathogeneic lymphocytes. Cyclophosphamide was added to the mobilization not only to enhance the yield of progenitor cells, but also to limit the risk of disease flare following G-CSF administration. We also chose not to add any posttransplant immunosuppressive agent routinely, to avoid interference with the interpretation of clinical sequelae. In stead a wait-and-watch policy was adopted.

From a technical viewpoint the treatment steps appeared feasible in all patients. The consecutive procedures of the treatment were well tolerated by most patients. No unexpected major toxicity or treatment related mortality occurred, although in several patients infectious complications neccessitated extra hospital admissions for parenteral antibiotic treatment. Hematological recovery was uneventful in all patients, showing an inverse relationship with the age of the patient. Longlasting lymphopenia was observed, which could mainly be attributed to slow recovery of naive $CD4^+$ T lymphocytes. With respect to efficacy, mobilization resulted in transient amelioration of disease activity in 5/14 patients (defined as ACR20 before conditioning), which was reinforced by the intensification of conditioning and transplantation procedures. In 8/12 of the patients clinical meaningful improvements (defined as good response according to EULAR response criteria) were recorded in more than 50% of follow-up visits. In 7 patients treatment with a DMARD (MTX 2, Leflunomide 5) was reinstituted because of relapse or persistent disease activity. This resulted in subsequent improvement in 3/7 patients. Interestingly, 2 patients had been refractory to these drugs (even in higher doses) before transplantation, suggesting that some degree of sensitivity to conventional drugs had been regained as observed by others as well [4]. Nevertheless, 4/12 patients failed to improve.

The individual clinical response at 3 months was found to be predictive for the subsequent disease course. These disease courses displayed a dichotomous pattern, enabling categorization in 'responders' and 'nonresponders'. Nonresponders did not differ from responders with respect to disease or patient related variables, such as age, disease activity and duration, previous therapy, presence of rheumatoid factor, although the numbers of patients may have been too low to detect such predictive factors. The observation of clearly divergent disease courses in the patients treated could reflect heterogeneity of disease processes. The observed differences in total IgG at baseline and after therapy could be indicative of this. The clinical results of our study are in line with those reported previously from single-center pilotstudies where different regimens were employed. Long term remissions as well as relapses and progressive disease have been reported with the various regimens used, both in rheumatoid arthritis and other autoimmune diseases. Our data do not allow definitive conclusions on whether the immunological effects of the treatment are only quantitative ('debulking' of inflammatory load) or qualitative as well (e.g. tolerization of pathogenic T lymphocytes). From a T-cell centered perspective it might be inferred from the present study that not all pathogenic T lymphocytes were eradicated or that some had been reinfused with the graft. In fact, such mechanisms clearly can be operative after intense immunosuppression and autologous stem cell transplantation [14]. This would imply that remissions can only be achieved by further intensification, e.g. by in vivo T cell depletion. Clearly, this could add to the toxicity. The same would be anticipated when myeloablative regimens in combination with either autologous or allogeneic stem cell transplantation were employed. From the patient's and treating physician's perspective, responses were clinically meaningful in a number of patients with resultant enhanced quality of life (data not shown). It remains to be proven that any superior efficacy of a more rigorous approach will compensate for increased toxicity in terms of quality-adjusted-life-expectancy [15]. Adequate assessment of risk/benefit requires properly designed and conducted prospective randomized controlled trials with a long follow-up. Such studies will need to be multicenter given the low expected rate of recruitment of patients, due to the recent introduction of effective less toxic antirheumatic therapies such as anti-TNF blocking agents and leflunomide. The key issue to be addressed in our opinion is whether a brief period of intense immune suppression aimed at immunoablation is superior to continuous moderate immune suppression in terms of safety, tolerability and efficacy, and whether these effects can be maintained during a longer follow-up.

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