The influence of a partially HLA-matched blood transfusion on the disease activity of rheumatoid arthritis


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Abstract

Objective. Based on the immunosuppressive effects of blood transfusions in organ transplantation, we determined the effect of a blood transfusion on disease activity of rheumatoid arthritis (RA).

Method. In this double-blind pilot study, 40 patients with active RA were randomly assigned to receive a HLA-DRB1-matched blood transfusion (n = 30) or placebo (n = 10). Disease activity was scored according to the American College of Rheumatology response criteria during 6 months of follow-up.

Results. After 1 month and 6 months, respectively, 6 and 16% of patients fulfilled the response criteria in the blood transfusion group compared to none and 30%, respectively, in the placebo group. Following correction for the increase in haemoglobin levels, a majority of the response parameters in the blood transfusion group showed significant improvement compared to the placebo group.

Conclusion. A DRB1-matched blood transfusion shows improvement of symptoms in several RA patients. Additional studies are required to identify blood transfusion regimens that enhance the potential for therapeutic responses.

Key words: HLA match, Blood transfusion, Rheumatoid arthritis.

Rheumatoid arthritis (RA) is an autoimmune disease frequently treated with immunosuppressive drugs. Blood transfusions were reported to have immunosuppressive effects and might thus be beneficial to RA patients.

Blood transfusion, when given to the recipients prior to renal transplantation, was found to prolong allograft survival [1]. If the blood was matched for one HLA-DR antigen between blood donor and acceptor, the chance of rejection of the graft [2] was even further reduced. In addition, in some cases of recurring spontaneous abortions, a beneficial effect of blood transfusions was found [3]. In other immune-mediated conditions, like Morbus Crohn, a longer remission of the disease was observed after perioperative blood transfusions [4, 5]. Clinical improvement following operations with blood transfusions was also observed in RA. In addition, several uncontrolled studies reported a beneficial effect of blood transfusions on arthritis activity in RA [6–9].

The present double-blind, placebo-controlled, pilot study aims to investigate whether one blood transfusion matched for one HLA-A, -B and -DR antigen, and mismatched for the other HLA-A, -B and -DR antigen, given as an adjuvant therapy, diminishes disease activity of RA.

Patients and methods

Patients with RA (according to the 1987 ARA criteria [10]) were included if (1) they had not received previous blood transfusions, (2) showed at least six painful or swollen joints according to the 28 joint count, and (3) the erythrocyte sedimentation rate (ESR) was at least 28 mm. Women who wished to become pregnant were excluded.

Forty patients were included: 30 were randomly assigned to a blood transfusion and 10 patients received...
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a placebo infusion. The patient, as well as the trained observer who studied the patient after the transfusion, were blinded for the intervention by covering both the units of fluid and i.v. lines during the procedure as well as the results of the blood tests.

Clinical and demographic data were documented at study entry.

All patients were typed for ABO, rhesus, Kell, Duffy and Kidd blood groups, for HLA class I (A, B and C), DR and DQ by serological methods, and for class II by polymerase chain reaction (PCR)-SSO and PCR-SSP; DRB1 PCR-SSP subtyping was performed in the case of DR4 and DR6. Moreover, serum was screened for irregular antibodies, and for HLA-A, -B and -C antibodies.

All 40 patients were assessed by a trained observer at study entry (visit 1), and 2 weeks (visit 2), 1 month (visit 3), 3 months (visit 4) and 6 months (visit 5) after the transfusion. The patients were monitored for the medication used and additional medical treatment. At every visit, the 28 joint count for swelling and pain (including the Ritchie score), ESR and a full blood count were obtained. Also, a Health Assessment Questionnaire (HAQ) and visual analogue scales (VAS) of morning stiffness, disease activity and pain were completed at every visit by the patient as well as a global assessment of the disease activity by the investigator.

The medication, including anti-rheumatic drugs, had to be stable 3 months prior to study entry and was continued at the same dosage during 6 months after the transfusion. A change of anti-rheumatic drugs or interventions considered necessary because of sustained RA disease activity during the 6 months of follow-up was considered as a failure of the therapy.

The study was approved by the medical ethical committee of the hospital.

Blood transfusion: product specification and HLA matching

Thirty patients received one unit (250 cm$^3$) of erythrocytes with a buffy coat containing $10^9$ leucocytes which was donated maximally 24 h prior to transfusion.

Apart from matching for ABO and rhesus-D compatibility, the donor was also selected to be compatible at rhesus, Kell, Duffy and Kidd antigens. The HLA data of the patients who were randomized for the blood transfusion were compared to the HLA data of the blood donors. A donor was carefully selected for each patient to have one HLA-A, -B and -DR (DR 1–18) antigen match, and one HLA-A, -B and -DR mismatch. Selected donors who carried either DR4 and/or DR6 were subtyped by the DRB1 PCR-SSO method. In all 30 cases, the patients had indeed received a blood transfusion mismatched on one HLA-A, -B and -DRB1 allele, and matched on the other HLA-A, -B and -DRB1 allele.

Ten patients received a placebo infusion of 250 cm$^3$ 0.9% natrium chloride.

Outcome parameters

The disease activity of RA was measured by outcome parameters as defined in the ACR 20% criteria [11].

Immunological monitoring

HLA alloantibodies were measured in each patient before and after the (blood) transfusion. This was tested by an ELISA technique which could detect HLA class I IgA, M and G antibodies. Positive sera were screened against a selected panel of lymphocytes to determine the HLA antibody specificities.

Statistics

The patients treated with placebo or blood transfusion were tested whether or not they met the ACR 20% response criteria. Apart from the combination of parameters in the ACR response criteria, every parameter was considered separately as well.

The follow-up of patients was according to the intention-to-treat analysis.

A $\chi^2$ test was performed in the case of dichotomous data. In the case of non-normally distributed data, the Kruskal–Wallis and Median tests were used. The change between the first visit and each follow-up assessment of the distinct variables was compared between both arms in a one-way ANOVA. In order to rule out the effect of the rise in haemoglobin as the cause of improvement, a covariance analysis of each parameter was performed in addition.

Results

Outcome of RA

No significant difference between the placebo group and the blood transfusion group was observed according to sex, age, disease duration and number of patients with erosive disease (Table 1). The number of patients with increased serum rheumatoid factor levels and nodular disease was higher in the blood transfusion group, but

<table>
<thead>
<tr>
<th>Outcome parameter</th>
<th>Blood (n = 30)</th>
<th>Placebo (n = 10)</th>
</tr>
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<tbody>
<tr>
<td>No. of females (%)</td>
<td>18 (60)</td>
<td>8 (80)</td>
</tr>
<tr>
<td>Median age (range), yr</td>
<td>59 (35–86)</td>
<td>58 (51–70)</td>
</tr>
<tr>
<td>Median disease duration (range), yr</td>
<td>7 (0.3–49)</td>
<td>8 (1–20)</td>
</tr>
<tr>
<td>RF positive (%)</td>
<td>23 (77)</td>
<td>9 (90)</td>
</tr>
<tr>
<td>Erosive (%)</td>
<td>24 (80)</td>
<td>8 (80)</td>
</tr>
<tr>
<td>Nodular (%)</td>
<td>13 (43)</td>
<td>3 (30)</td>
</tr>
<tr>
<td>Median number of DMARDs used</td>
<td>2 (0–8)</td>
<td>2 (1–7)</td>
</tr>
<tr>
<td>DMARD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sulphasalazine (%)</td>
<td>11 (37)</td>
<td>4 (40)</td>
</tr>
<tr>
<td>Methotrexate (%)</td>
<td>7 (23)</td>
<td>1 (10)</td>
</tr>
<tr>
<td>Antimalarial (%)</td>
<td>4 (13)</td>
<td>1 (10)</td>
</tr>
<tr>
<td>Prednisone (%)</td>
<td>1 (3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Other</td>
<td>5 (17)</td>
<td>3 (30)</td>
</tr>
<tr>
<td>No DMARD</td>
<td>2 (7)</td>
<td>1 (10)</td>
</tr>
</tbody>
</table>
this difference was not significant. The use of disease-modifying anti-rheumatic drugs (DMARDs) in both groups was similar, as well as the percentage of patients without DMARDs (Table 1). Also, the baseline characteristics of disease activity and haemoglobin level were equal in both groups at entry to the study (Table 2).

Two patients in the placebo group were considered failures: one because a DMARD was started 2 weeks after the infusion and one because of a hospital admission for orthopaedic surgery 3 months after the infusion. Eight patients receiving the blood transfusion had a treatment failure. All received new DMARD therapy within 6 months after the transfusion: two started after 2 weeks, one after 3 months, and five between 3 and 6 months after the transfusion.

In the blood transfusion group, one (3%) patient responded to the ACR 20% criteria after 2 weeks, two (6%) patients after 1 month, five (20%) after 3 months and five (20%) after 6 months (Fig. 1). In the placebo group, no patients responded after 2 weeks and 1 month, one (10%) after 3 months and three (30%) after 6 months of follow-up (Fig. 1). The differences in the percentage of patients responding to the ACR criteria between the groups treated with placebo or blood transfusion were not significant. Logistic regression of the patients fulfilling these criteria during the course of the observation period did not show a significant difference between the placebo and blood transfusion group ($P = 0.28$).

None of the individual patients met the response criteria at more than two visits. Only one out of the four patients (including one from the placebo group) who showed a response at more than one visit responded within 1 month after transfusion. Nine patients (including two from the placebo group) showed a response at only one visit.

The individual disease activity parameters showed a larger decrease in the blood transfusion group compared to the placebo group for ESR, HAQ, disease assessment according to the doctor, and the Ritchie score (Fig. 2). In contrast, the global disease activity score according to the patient decreased more in the placebo-treated patients. After correction for the increase in haemoglobin levels, the statistical significance of the decrease in ESR in the blood transfusion group disappeared ($P = 0.84$; Table 3). The beneficial effect of the blood transfusion on the HAQ score and global disease assessment according to the patient remained significant after correction for the raised haemoglobin levels, and the larger decrease in Ritchie score and VAS pain score of the patient in the blood transfusion group became significant. The larger improvement of the global disease assessment according to the doctor in the placebo group also remained significant (Table 3).

### Immunological parameters

No donor-specific HLA class I antibodies were detected in the placebo group. Seven patients (23%) showed donor-specific antibodies after blood transfusion (Table 4). In three cases, the donor-specific antibodies were associated with an improvement of RA according to the ACR criteria.

### Discussion

This is the first placebo-controlled and double-blind study on the effect of a blood transfusion on disease activity in RA. Given the experiences in organ transplantation, blood was used with one HLA-A, -B and -DR antigen match (with a mismatch for the other HLA-A, -B and -DR antigen) between donor and recipient.

Within 1 month after the blood transfusion, two patients fulfilled the ACR response criteria, with none in the placebo group. After 3 and 6 months, the percentage of patients who fulfilled the ACR 20% response criteria in the groups who received a blood transfusion and the placebo did not differ significantly. Several individual disease activity parameters, including the ESR, HAQ, Ritchie score and pain score according to the patient, showed a stronger improvement following the blood transfusion. Following correction for the...
Fig. 2. Comparison of the mean change in ESR, Health Assessment Questionnaire (HAQ), the Ritchie score, and the visual analogue score of pain of the patient between patient groups that received an infusion with either blood or placebo. The means and 95% confidence intervals are depicted.

Table 3. Mean differences (delta) of the change in the outcome parameters of the placebo group minus the blood transfusion group after adjustment for the haemoglobin correction

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Delta</th>
<th>(s.e.)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESR</td>
<td>0.28</td>
<td>(1.38)</td>
<td>0.84</td>
</tr>
<tr>
<td>HAQ</td>
<td>0.20</td>
<td>(0.06)</td>
<td>0.002</td>
</tr>
<tr>
<td>Patient’s disease activity</td>
<td>2.04</td>
<td>(0.49)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Doctor’s disease activity</td>
<td>-1.18</td>
<td>(0.45)</td>
<td>0.01</td>
</tr>
<tr>
<td>Ritchie score</td>
<td>4.01</td>
<td>(1.88)</td>
<td>0.03</td>
</tr>
<tr>
<td>VAS pain</td>
<td>0.58</td>
<td>(0.30)</td>
<td>0.05</td>
</tr>
<tr>
<td>Swollen joint count</td>
<td>-1.28</td>
<td>(0.88)</td>
<td>0.15</td>
</tr>
</tbody>
</table>

A positive value shows more improvement in the blood transfusion group compared to the placebo group and a negative value shows an outcome in favour of the placebo group. S.E. = standard error.

Table 4. Number of patients with a HLA class I antibody response after the transfusion in relation to improvement of RA according to the ACR response criteria

<table>
<thead>
<tr>
<th>HLA antibody reaction</th>
<th>Placebo (n = 10) Improvement</th>
<th>Blood transfusion (n = 30) Improvement</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Negative</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Non-specific</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Donor specific</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Change in haemoglobin levels, the significance of the ESR change disappeared and stronger improvement of the HAQ and Ritchie score in the blood transfusion group remained significant. These findings justify the conclusion that a blood transfusion had a mild anti-inflammatory effect on RA. The study was not designed to answer the question whether leucocytes and/or HLA matching were responsible for this effect. Moreover, with the HLA-typing techniques used, it is not possible to identify every HLA subtype, so specific matching (and mismatching) can only be based on the available methods.

The influence of HLA matching of the leucocytes in the case of a desired immunosuppressive effect was illustrated by a higher percentage of renal allograft survival after a HLA-A and -B compatible blood transfusion compared to a random blood transfusion [12]. Later, it was demonstrated that the percentage of renal allograft 5 yr survival was increased by a pre-transplant transfusion of one unit of fresh blood, containing 10^9 leucocytes and matched on one DR antigen (81%), compared to a blood transfusion without DR matching (57%) or no transfusion (45%) [2]. The number of rejection episodes was also reduced after a one DR antigen-matched blood transfusion in renal transplants and heart transplants [2, 13].
The mechanism by which the blood transfusion matched for one DR antigen has a long-lasting beneficial effect on renal graft survival remains unclear. It can be hypothesized that host T cells, which recognize ‘autologous’ class II antigens on leucocytes of the blood donor, are downregulated, either by the induction of T-suppressor cell or by the deletion of cytotoxic T cells directed against these ‘autologous’ antigens [2, 14–16].

The observation that RA improves after a blood transfusion was first described in 1931 by Copeman [17]. A ‘dramatic’ improvement of pain and swollen joints was also described later after a blood transfusion from pregnant as well as from non-pregnant donors [8, 9]. Others [6], who treated patients with refractory anaemia with fresh whole blood (250 cm$^3$ weekly for 3–5 weeks), also observed improvement of RA activity. In contrast with these observations, Simpson et al. [7] did not find differences in RA activity between 40 patients who received packed cells or whole blood, 500 ml twice, and 50 patients who were not treated with a transfusion, in an open study. These studies suggest that blood transfusions are beneficial in active RA, but the open design of these studies inhibits definitive conclusions.

Other studies have observed an anti-inflammatory effect induced by the administration of plasma compounds or by transfused leucocytes. Anti-rheumatic effects were described by the parenteral administration of serum, including serum obtained from placental blood, but these observations could not be confirmed by all investigators [18–24]. The hypothesis that leucocytes are the immunosuppressive agents of the blood transfusion in RA is supported by a study [25] on the administration of 30–250 x 10$^6$ mononuclear white blood cells at 6 week intervals. Six out of 11 patients treated as such and who discontinued treatment with DMRADS improved according to the ACR 20% response criteria [25]. More improvement was observed in patients receiving a greater number of cells than in those receiving fewer cells.

The improvement in disease activity of RA after a blood transfusion matched for one HLA-A-, one -B and one -DR antigen, and mismatched for the other HLA-A, -B and -DR antigen, as observed in the present study, does not allow a conclusive statement of efficacy. The percentage of patients who fulfilled pre-defined response criteria is not superior to placebo treatment. However, this trial was inadequately powered to demonstrate efficacy and four out of the seven efficacy parameters measured improved significantly, even after correction for the increased haemoglobin levels, suggesting a small anti-inflammatory or immunosuppressive effect. It should be realized that the continuation of anti-rheumatic drugs, the unusually high placebo response and the low number of patients have restricted the chance of finding a difference in responders between placebo- and blood-treated RA patients. Furthermore, the optimal dosage of the infused blood to obtain an immunosuppressive effect previous to transplantation remains to be determined. In some countries like the UK, however, these kinds of studies cannot be performed because the white blood cells have to be removed from the transfused blood because of the theoretical risk of Creutzfeld–Jacob disease. Nevertheless, we conclude that the results of the present study support further attempts to develop anti-rheumatic therapies by means of immune deviation through the administration of partly matched allogenic HLA antigens.

References


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