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Late conversion from steroids to azathioprine in cyclosporin-treated renal graft recipients

G. Offermann[1], P. H. Krause[2], D. Johann[1], H.-H. Neumayer[1], J. Scholle[1], and A. Schwarz[1]

[1] Klinikum Steglitz, Freie Universität Berlin, Hindenburgdamm 30, D-1000 Berlin 45
[2] Pathologisches Institut, Krankenhaus Neukölln, Rudowerstrasse 48, D-1000 Berlin 47

Abstract. In renal graft recipients primarily treated with cyclosporin and low-dose methylprednisolone, withdrawal of the long-term steroid medication increases the likelihood of developing rejection episodes. In order to determine the predictive value of clinical parameters and routine prewithdrawal graft biopsies for the risk of rejection, the authors studied 141 kidney recipients from whom steroids were withdrawn 7-9 months after transplantation in a clinically stable situation. Both the quality of the HLA-match and the results of prospective graft biopsies were found to correlate significantly to the occurrence of acute rejection. In order to investigate the influence of additional azathioprine medication on the incidence of acute rejections in recipients not receiving steroids, immunosuppression was continued with cyclosporin monotherapy in 88 patients and with cyclosporin plus azathioprine in 53 patients. The risk of developing rejection episodes was significantly reduced from 48% after 1 year on monotherapy to 28% after the addition of azathioprine medication.

Key words: Conversion from steroids to azathioprine, in kidney transplantation - Azathioprine conversion after steroids, in kidney transplantation - Acute rejection after conversion, in kidney transplantation - Cyclosporin and conversion, in kidney transplantation.

The combination of cyclosporin and low-dose corticosteroids is widely accepted as a standard immunosuppressive regimen in the early phase after renal transplantation [1, 5, 7]. However, in order to avoid cyclosporin-induced nephrotoxicity [10, 15, 18] and/or the side effects of steroid medication [3, 9], changes in this protocol have been proposed for the long-term treatment of kidney graft recipients. Thus far, two alternative approaches - conversion from cyclosporin to azathioprine and withdrawal of steroids - have both been reported to trigger rejection episodes in some patients with stable graft function [2, 4, 15].

In the present study, kidney recipients with stable graft function for more than 6 months after transplantation and from whom steroids were withdrawn were investigated. The study was designed to determine whether the risk of acute rejection without steroids can be predicted by clinically available data or by routinely performed prewithdrawal graft biopsies and whether the incidence of rejection episodes can be influenced by azathioprine after cessation of steroid medication.

Patients and methods

The investigation was performed as a single center study. All patients received a kidney transplantation following a standard surgical procedure and the same initial immunosuppressive regimen of cyclosporin and low-dose methylprednisolone.

Cyclosporin was administered twice daily with an oral starting dosage of 12 mg/kg body weight per day. The dosage was adjusted according to whole blood cyclosporin trough levels measured by radioimmunoassay using a polyclonal, nonspecific antiserum (Sandoz RIA kit). The cyclosporin trough levels were kept in the range of 400-800 ng/ml during the first 6 weeks after transplantation and in the range of 200-400 ng/ml thereafter.

Methylprednisolone was tapered from an initial daily oral dose of 32 mg to 4 mg at 6 months after transplantation. Once the graft function had become stable for at least 4 weeks on 4 mg methylprednisolone per day and the serum creatinine was below 250 μmol/l, the steroid medication was withdrawn. This occurred 7-9 months after transplantation.

Of the 256 consecutively transplanted renal allograft recipients [239 cadaveric donors (CD), 17 living related donors (LRD)] treated according to this protocol and observed for a minimum of 12 months after transplantation, 115 (45%) were never taken off of steroids. The reasons for this, and for excluding them from further analysis, included acute rejection episodes within 4 weeks on 4 mg methylprednisolone per day ($n = 31$), serum creatinine above 250 μmol/l ($n = 9$), early conversion from cyclosporin to azathioprine due to unsatisfactory graft function ($n = 9$), graft loss ($n = 30$), death of the patient during the first 7 months after transplantation ($n = 17$), and the patient's informed decision ($n = 19$). Thus, the final study group consisted of 141 patients (126 CD, 15 LRD). All had serum creatinine below 250 μmol/l and stable graft function on 4 mg methylprednisolone for at least 4 weeks,

Offprint requests to: G. Offermann

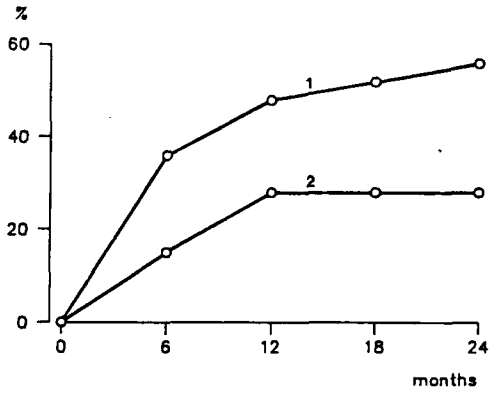

Fig. 1. Probability of developing rejection episodes after cessation of methylprednisolone treatment (month 0) in kidney graft recipients on cyclosporin monotherapy (CyA-Mono) or cyclosporin plus 50 mg azathioprine per day (CyA-AZA). *Curve 1:* CyA-Mono, $n = 88$; *curve 2:* CyA-AZA, $n = 53$; $P < 0.005$

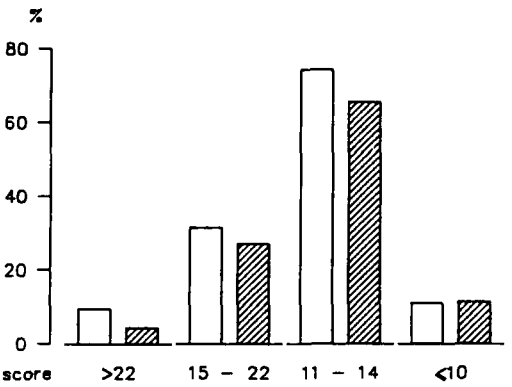

Fig. 2. Quality of donor/recipient HLA-match according to a scoring system in kidney recipients ($n = 141$) on cyclosporin treatment who remain stable or reject after withdrawal of steroid medication 7-9 months after transplantation. Score: DR = 7; B = 4; A = 1. □, stable; ▨, rejecting; $P < 0.025$

and steroids were withdrawn from all patients 7-9 months after transplantation. In 100 patients a routine graft biopsy was performed 1-7 days before methylprednisolone treatment was discontinued.

Immunosuppressive therapy was continued with cyclosporin alone in the first 88 patients (78 CD, 10 LRD) who entered the study; the following 53 patients (48 CD, 5 LRD) received cyclosporin plus 50 mg azathioprine per day. There were no significant differences between these two treatment groups with respect to preformed cytotoxic antibodies, number of retransplants or previous rejections, mean number of HLA-mismatches, or mean number of pretransplant blood transfusions (Table 1). All patients had received at least three transfusions prior to transplantation. Cyclosporin blood trough levels were closely kept in the range of 200-400 ng/ml in both groups, and there was no difference in the mean levels between the two groups either at the time of steroid withdrawal (260 vs 245 ng/ml) or during the follow-up period.

The standard dose of 50 mg azathioprine, regardless of body weight, was used to avoid splitting of tablets. Thus, patients in the cyclosporin plus azathioprine group received between 0.7 mg/kg and 1.0 mg/kg azathioprine per day.

Graft function was monitored at weekly intervals during the 1st month after steroid withdrawal and at monthly intervals thereafter in the transplant centers' outpatient ward. Acute graft rejection was suspected when the serum creatinine increased by more than 20% of the previous level. In these instances a graft biopsy was performed. Other causes for the rise in creatinine were excluded on the basis of histological findings (recurrence of original disease, de novo glomerulonephritis, cyclosporin nephrotoxicity), ultrasound investigation (postrenal obstruction), urinary bacteriology, cyclosporin blood levels, and by checking for potentially nephrotoxic medication. The diagnosis of acute rejection was based on the results of the graft biopsies. In case of rejection, the patient was treated with short-term, high-dose methylprednisolone and was kept on 4 mg per day thereafter.

Predictive parameters

To determine the predictive value of clinical parameters for the risk of rejection without steroids, the following information was obtained:

1. Quality of HLA-match: The number of HLA-mismatches in the A-, B-, and DR-loci, as well as the combined number of mismatches for B + DR and for A + B + DR, was counted in each patient and evaluated in the patient subgroups by chi-square distribution statistics. In order to account for the different effects of HLA-A, -B or -DR-matches on the results of renal transplantation [12, 16], a scoring system [11] was used in which a donor/recipient DR-match scored a 7, a B-match scored a 4, and an A-match scored a 1. The total match score was used as a nonparametric indicator of match quality.
2. Presensitization: The level of preformed cytotoxic HLA antibodies before transplantation and the number of previous graft losses due to rejection were used as indicators of presensitization.
3. Early course of transplantation: The number of rejection treatments before methylprednisolone was withdrawn and the serum creatinine at this time were used to assess the early clinical course.
4. Graft histology: The biopsy specimens obtained routinely before stopping the steroid medication or when rejection was clinically suspected were all examined by one of the authors (P.H.K.). The findings in light microscopy were classified as given in Table 3; scores of 0 to + + + were used for each histological parameter. The term "mononuclear cell infiltrates" was used to describe findings with cell infiltrates that could not be diagnosed as acute or chronic transplant nephritis. The term was necessary because minimal cell infiltrates were often the only abnormal finding in these biopsies from clinically stable recipients. Acute nephritis was defined as significant interstitial cell infiltrates plus edema and chronic nephritis as cell infiltrates plus increased fibrous tissue. The terms "diffuse" and "striped" fibrosis described an increase in fibrous tissue without cell infiltrates occurring in either a generalized (diffuse) or regional (striped) form. Immunohistological staining for IgG, IgM, IgA, C3 complement, and fibrine was only considered positive when vascular (nonglomerular) lesions were encountered.

Data analysis

In order to assess the time-dependent probability of acute rejection without methylprednisolone and to compare the two treatment protocols, the life table method and log rank test [14] were used. Comparisons of predictive parameters for the risk of rejection were performed by two-tailed tests, including the chi-square test and the Mann-Whitney U-test.

Results

Graft rejection

The global results for all patients transplanted during the study period were 81.6% graft survival at 1 year and 76.8% at 2 years; patient survival was 94.0% at 1 year and 91.9% at 2 years. All graft losses or patient deaths occurred in the recipients excluded from the study, i.e., those from whom the steroid medication was never withdrawn. For the recipients included in the study, the observation time after withdrawal of methylprednisolone ranged from 3 to 50 months. In order to account for the time dependency of the incidence of rejection episodes, the cumulative risk of rejection was calculated (Fig. 1). The patients on cyclosporin monotherapy had a significantly ($P < 0.005$) higher risk of developing acute rejection without steroids than those on cyclosporin plus azathioprine. During the 1st year, acute graft rejection was observed in 48% of the patients on monotherapy and in 28% of those on cyclosporin plus azathioprine. The rejection episodes tended to occur earlier in the monotherapy group than in the azathioprine group. With monotherapy 48% of the acute rejections observed during the 1st year developed during the first 3 months and 75% during the first 6 months. In the azathioprine group, the respective figures were 32% at 3 months and 53% at 6 months. The rejection episodes responded to methylprednisolone treatment in all cases and no graft losses occurred during the study period. Thus, at the end of the study, 1- and 2-year graft and patient survival was 100% in both treatment groups. Four patients from the cyclosporin monotherapy group, however, developed chronic rejection during the following months and lost their graft function 1-3 years later. Only a slight, and not uncommon, increase in serum creatinine between the time steroids were stopped and the end of observation was recorded more frequently ($P < 0.05$) in the patients who developed rejection (Table 2).

Predictive parameters

The quality of HLA-matches between donor and recipient was significantly better in patients who proved not to be steroid-dependent in the late phase after kidney transplantation (Fig. 2). A tendency to a lower number of HLA-mismatches in the A-locus alone and in the combination of B + DR-loci was observed in patients with stable graft function without steroids; however, this did not reach statistical significance. This difference in match quality between stable and rejecting patients became significant in the chi-square distribution test when the combined number of mismatched HLA-A + B + DR antigens was counted ($P < 0.05$). It became even more pronounced when the different effects of A-, B-, and DR-matching on transplantation results were taken into account using the scoring system and the nonparametric U-test ($P < 0.025$).

No significant differences between stable and rejecting recipients were found for the level of preformed HLA antibodies, the number of previous graft losses due to rejection, or the number of rejection treat-

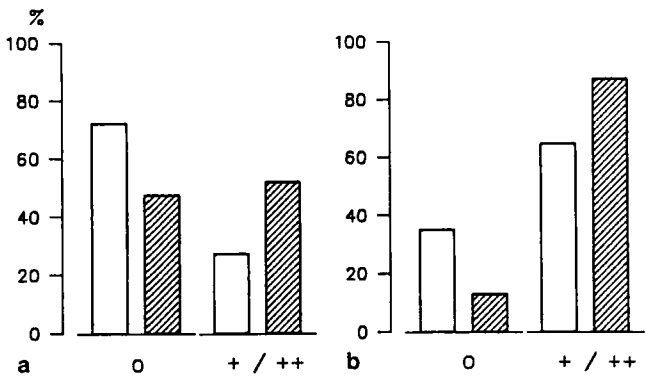

Fig. 3. Incidence of interstitial signs of low-grade rejection in prospective renal graft biopsies obtained before withdrawal of steroids. □, patients remaining stable without steroids ($n = 51$); ▨, patients developing rejection without steroids ($n = 49$). **a** Chronic interstitial nephritis; $P < 0.001$. **b** Mononuclear cell infiltrate; $P < 0.05$

Table 1. Comparison of treatment groups according to risk factors for rejection. CyA, Cyclosporin; AZA, azathioprine

	CyA alone ($n = 88$)	CyA + AZA ($n = 53$)
Cytotoxic HLA antibodies		
> 50%	2%	2%
> 80%	-	-
Second transplants	11%	12%
Mean number of mismatches (HLA-A + B + DR)	2.3	2.5
Mean number of pretransplant blood transfusions	3.6	3.3
CyA levels at time of steroid withdrawal (ng/ml)	260	245
Previous rejection episodes per patient	0.36	0.5

Table 2. Increase in serum creatinine (Δ creatinine) from time of steroid withdrawal to end of observation in kidney recipients who remained stable ($n = 77$) or who developed rejection ($n = 64$) without steroids ($P < 0.05$)

Δ Creatinine	Stable recipients	Rejecting recipients
None	56%	22%
< 25 μmol/l	33%	32%
< 50 μmol/l	5%	15%
< 75 μmol/l	3%	9%
> 75 μmol/l	3%	22%

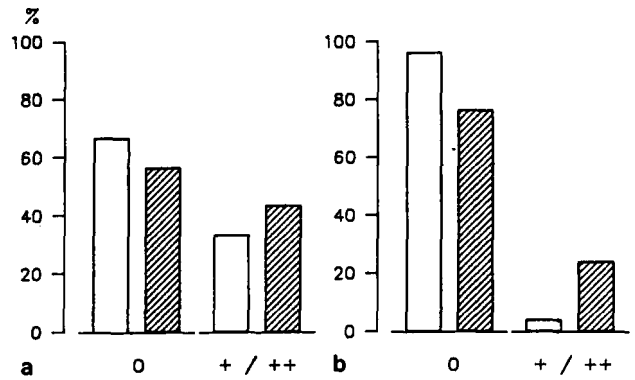

Fig. 4. Incidence of vascular signs of clinically asymptomatic rejection in prospective renal graft biopsies obtained before withdrawal of steroids. □, patients remaining stable without steroids ($n = 51$); ▨, patients developing rejection without steroids ($n = 49$). **a** Sclerosing vascular lesions; $P < 0.025$. **b** Vascular immune complexes; $P < 0.05$

ments before withdrawal of steroids. Because of the transplant centers' rather uniform pretransfusion policy throughout the study period, the mean number of blood transfusions prior to transplantation was nearly identical in both subgroups (3.4 vs 3.5).

The results of 100 prospective routine graft biopsies obtained at the time of steroid withdrawal are summarized in Table 3. Of the histological parameters of interstitial graft rejection, mononuclear cell infiltrates and chronic graft nephritis occurred significantly more often in patients who developed rejection without methylprednisolone (Fig. 3). Of the vascular rejection parameters, the same held for chronic sclerosing vasculopathy and positive immunoglobulin (mostly IgM) staining (Fig. 4). Although fibrosis as a single entity only tended to occur more frequently in the rejecting recipients, the higher incidence of striped fibrosis in this patient group reached borderline significance. There was no difference between the two groups of recipients in the occurrence of tubular changes (cell necrosis, vacuolization, megamitochondria, microcalcification) or glomerular changes.

Discussion

The withdrawal of corticosteroids from cyclosporin-treated renal graft recipients has been shown to increase the risk of rejection episodes [3]. Although rejections in this situation usually respond to steroid treatment [4, 9], the potential decrease in, or even loss of, graft function in some patients has to be weighed against the advantages of avoiding long-term steroid medication in other patients. Thus, it would be desirable to be able to predict whether an individual patient would benefit from steroid withdrawal or whether he/she should be kept on corticosteroids.

The present study included only patients with good graft function and a seemingly stable immunological situation. This probably explains why factors known to have a negative influence on the overall results of renal transplantation, such as preformed cytotoxic antibodies and retransplantation, did not influence the likelihood of rejection without steroids. For the same reason, the incidence of rejection episodes in the early phase after transplantation could not be used to predict graft stability on cyclosporin monotherapy.

After conversion from cyclosporin to azathioprine, no effect of the HLA-match on the development of rejection was reported [6, 19]. In contrast to these findings, graft stability without steroids correlated significantly with the quality of the HLA-match in the present study. Although this observation may be an interesting extension of the known influence of HLA-matching on renal graft survival [13], the HLA-match has only little predictive value due to the large overlap between stable and rejecting patients.

The most conclusive results were obtained from the graft biopsies performed routinely before cessation of steroid treatment. Although the patients were

Table 3. Results of prospective routine graft biopsies in 100 kidney recipients remaining stable without steroids ($n = 51$) or rejecting later on ($n = 49$). The histological findings were graded negative (0), mild (+), medium (++), or severe (+++)

	Stable recipients ($n = 51$)				Rejecting recipients ($n = 49$)				
	0	+	++	+++	0	+	++	+++	
Interstitial lesions									
Acute nephritis	45	4	2	–	38	6	4	–	NS
Chronic nephritis	37	8	4	2	22	17	8	2	$P < 0.001$
Mononuclear cell infiltrates	18	27	6	–	6	32	11	–	$P < 0.05$
Diffuse fibrosis	48	3	–	–	39	7	3	–	NS
Striped fibrosis	32	18	1	–	22	22	5	–	NS ($P < 0.1$)
Vascular lesions									
Intima proliferation	41	9	–	1	39	6	4	–	NS
Vascular sclerosis	34	17	–	–	26	15	8	–	$P < 0.025$
Immunohistological staining	49	2	–	–	26	9	3	–	$P < 0.05$

in a clinically stable situation at the time of the biopsy, histological examination frequently revealed signs of mild rejection. That is, chronic interstitial nephritis and/or sclerosing vascular lesions were mostly found in patients who later developed acute rejection episodes without steroids. Again, the predictive value of graft histology for the individual patient is limited. Some patients with negative histology nevertheless developed rejection and vice versa. The results of the prospective biopsies suggest excluding patients with medium grade interstitial or vascular findings from steroid withdrawal in order to reduce the risk of rejection. A similar strategy for obtaining a preconversion biopsy has been proposed for conversion from cyclosporin to azathioprine [17].

Clearly the most significant finding of this study was the impact that azathioprine had when it was introduced into the immunosuppressive regimen after steroids were stopped. This is in accordance with Kupin et al. [8], who already reported on 14 patients with a similar protocol and a low, short-term incidence of rejection when azathioprine replaced methylprednisolone.

The risk of rejection after changes in the treatment protocol depends largely on observation times. Calculation of the time-dependent cumulative risk of rejection revealed that more than half of the patients originally on cyclosporin monotherapy were back on methylprednisolone 2 years after steroid withdrawal. By replacing steroids with azathioprine, the proportion of steroid-free patients after 2 years increased to about 70%. The incidence of rejections is most likely influenced by the time of steroid withdrawal and by the cyclosporin blood levels thereafter [8, 9]. In the present study, the immunosuppressive protocol was changed relatively early and the cyclosporin levels were kept in a lower range.

Thus, it may be concluded from this study and from the literature that withdrawal of steroids substantially increases the likelihood of the patient's developing rejection episodes. The risk can be reduced by performing a graft biopsy prior to changes in treatment, by introducing azathioprine into the immunosuppressive regimen, by maintaining cyclosporin levels in a medium range, and by the late withdrawal of steroids.

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