Initial Treatment of Idiopathic Nephrotic Syndrome in Children: Prednisone versus Prednisone Plus Cyclosporine A: A Prospective, Randomized Trial

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on behalf of the Arbeitsgemeinschaft für Pädiatrische Nephrologie (APN)*

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Previous studies of the Arbeitsgemeinschaft für Pädiatrische Nephrologie in children with steroid-sensitive nephrotic syndrome have shown that the length of initial prednisone therapy has an impact on the subsequent relapse rate. The aim of this randomized, prospective, multicenter study was to reduce the number of relapses further by increasing the initial immunosuppression: Patients with an initial attack of nephrotic syndrome were randomly allocated to treatment with 6 wk of 60 mg/m² per d prednisone followed by 6 wk of 40 mg/m² per 48 h (Pred group) or to the same prednisone treatment plus 8 wk of cyclosporine (Pred+CsA group). The primary end point was first relapse; follow-up was truncated at 2 yr. In the Pred+CsA group (n = 49 patients), the first relapse occurred later compared with the Pred group (n = 55 patients) (median 22.8 versus 12.5 mo). After 6 mo, 10.4% of patients in the Pred+CsA group experienced a first relapse versus 31.5% in the Pred group (P = 0.01); after 1 yr, 36.5 versus 51% (P = 0.15); and after 2 yr, 51 versus 50%. The mean relapse rate per patient was 0.12 versus 0.57 after 6 mo (P = 0.01), 0.63 versus 1.03 after 1 yr (P = 0.02), and 1.03 versus 2.06 after 2 yr (not significant). The significant benefit for adding CsA was lost after 9 to 12 mo. GFR remained unchanged. The subsequent treatment rate with cyclophosphamide was lower in the CsA group (five versus 12 patients) after 2 yr. With the use of logistic regression statistics, children who were younger than 7 yr show a significantly better sustained remission rate with initial CsA treatment for the 2-yr observation time (P = 0.03). It remains questionable, however, whether the intensified initial treatment with CsA could be recommended generally.


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ment of relapses and to assess the tolerability and the safety of the total immunosuppressive load as well as the specific CsA-associated side effects.

Materials and Methods

The design of the trial was a prospective, randomized, multicenter, open-label study. Inclusion criteria were pediatric patients (1 to 16 yr of age) with first manifestation of NS with proteinuria $>40$ mg/m$^2$ per h, a serum albumin concentration $<25$ g/L, and a preserved GFR (creatinine clearance $>68$ mL/min per 1.73 m$^2$); no previous treatment with corticosteroids or immunosuppressive agents; and no contraindication to corticosteroid therapy. Renal biopsy was not requested for admission to the study. Informed consent was obtained from the parents of the patients and from patients, depending on their perceptive capability. Exclusion criteria were low c3-complement; postinfectious glomerulonephritis; and systemic diseases such as lupus erythematoses, diabetes, amyloidosis, vasculitis, Schönlein-Henoch nephritis, metabolic or toxic nephritis, hepatitis B, or hereditary glomerular diseases.

Study Protocol

Patients with first manifestation of their NS were randomly assigned by the coordinating office (Medical School Hannover) in two groups: The first group received the standard treatment of 60 mg/m$^2$ per d prednisone for 6 wk followed by 40 mg/m$^2$ per 48 h for 6 wk (Pred group). The second group received the same prednisone regimen plus CsA in a dose of 150 mg/m$^2$ per d for 8 wk (Pred+CsA group). CsA was started when the urine was protein-free for 3 d. Patients who did not respond within 4 wk were defined as not steroid sensitive and were excluded from the study. For that reason, CsA has to be started within the first 4 wk of prednisone treatment and, therefore, fitted in the total duration of the initial 12 wk of prednisone treatment. Definitions and criteria for remission and relapses were the same as those used by the International Study of Kidney Disease in Children and the APN (15,16). Relapses were treated according to the APN regimen (60 mg/m$^2$ per d until urine was protein-free for 3 d followed by 40 mg/m$^2$ per 48 h for 4 wk). Patients who did not respond within 4 wk to prednisone treatment were excluded from the study and underwent a kidney biopsy. Patients with secondary steroid resistance also underwent a kidney biopsy and were excluded from the study. Patients with steroid dependency according to the APN definition were treated at the discretion of the investigator with cyclophosphamide (Figure 1).

The primary end point for the patients was the first relapse after initial treatment. For those who received cyclophosphamide, the start of cyclophosphamide treatment was the secondary end point of the study. Total follow-up was 24 mo and truncated at this point. Clinical data and laboratory values were reported to the central office at the following time points: Beginning of initial prednisone treatment and then after 6 wk, 12 wk, 6 mo, 12 mo, 18 mo, and 24 mo. Patients who were treated with CsA were monitored by measurement of CsA 12-h blood trough concentrations (mAb; Abbott, Wiesbaden, Germany) on days 3, 7, 14, 28, 42, and 56.

The ethics committees of each participating center approved the study protocol. Written informed consent was obtained from the parents of each patient before the study, which was performed in accordance with the Declaration of Helsinki. Serious adverse events and whether they might be treatment related and a reason for a change of protocol were discussed with the whole study group. No deviations were necessary during the course of the study.

Statistical Analyses

Data management and statistical analysis were performed independently by the Department of Biostatistics at the Medical School of Hannover by H. Geerlings. For an estimated difference of approximately 20% between both groups and 80% power for analysis, 40 cases had to remain in each arm for statistical comparison, for a difference of approximately 15%, 164 cases. Interim analyses were done for safety reasons. Statistical tests were done at the end of the study. The following tests were performed: Mann-Whitney U test, the paired and unpaired t test, the Wilcoxon signed rank test for life table analysis, and $\chi^2$. For the risk factor analysis, logistic regression, Cox regression, and receiver operator characteristics were applied. All procedures were carried out with the Statistical Package of Social Science (SPSS, version 11.0; SPSS, Inc., Chicago, IL).

Patients

A total of 152 patients were initially reported to the coordinating office; 76 were randomly assigned to the Pred group, and 76 were assigned to the group the Pred+CsA group. In both groups, some patients were excluded after randomization. The reasons for exclusions are listed in Figure 1. Eleven patients in the Pred group and 14 patients in the Pred+CsA group were withdrawn because they withdrew consent after randomization. Fourteen patients were excluded because the diagnosis later turned out not to be steroid-sensitive minimal-change disease. Five with focal segmental glomerulosclerosis were excluded in the Pred group and six with various types of glomerulonephritis in the Pred+CsA group. Two patients who developed varicella infections during initial treatment were excluded from the study for safety rea-
sons. They initially were randomly assigned into the Pred/H11001 CsA group but never received CsA because infections occurred before remission was achieved. The same holds true for one patient with a cerebrovascular thrombosis. Another patient developed a thrombosis in the internal carotid artery while on study medication. It was judged that this was not attributable to the study medication but to a kinking of his carotid artery. This case was reported in another publication (17). In total, 13% of the 152 patients were removed from the study for medical reasons and 18% for nonmedical reasons.

Demographic data of patients who remained in both arms of the study (Table 1) were comparable and were within the range of previous studies of the APN. Serum concentrations of total protein, albumin, cholesterol, creatinine, urea, magnesium, uric acid, IgA, IgG, and IgM were comparable in both groups and not statistically different. Before the end of the 2-yr observation period, 12 patients in the Pred group were treated at the discretion of individual investigators with cyclophosphamide because of frequent relapses with steroid toxicity or because of steroid dependency. In the Pred+/H11001 CsA group, five patients were treated with cyclophosphamide and did not complete the 2-yr follow-up. Therefore, the numbers of patients who remained for follow-up in the Pred group and the Pred+/H11001 CsA group after 6 mo were 53 versus 49, after 12 mo were 46 versus 45, after 18 mo were 45 versus 44, and after 24 mo were 43 versus 44.

Results

Mean CsA doses and achieved blood trough drug levels are shown in Table 2. Individual dose corrections were performed to keep patients’ CsA trough levels in the defined target range of 80 to 150 ng/ml. The mean CsA concentrations were within this range; however, some individuals show low values of 35 ng/ml and others high levels of 355 ng/ml. Neither side effects nor efficacy of the CsA treatment could be correlated to individual dosing and trough levels. CsA absorption characteristics were not measured in this study.

After completion of the initial treatment, the median cumulative sustained remission time in the Pred group was 12.5 mo (95% confidence interval [CI] 5.9 to 19.1 mo) and in the Pred+/H11001 CsA group was 22.8 mo (95% CI 11.6 to 34.0; \( P \lt 0.05 \)). The Kaplan-Meier curves for the cumulative sustained remission rate showed a delay of the first relapse in the Pred+/H11001 CsA group (Figure 2). Six and 12 mo after initial treatment, the differences between both treatment groups were significantly different \( (P < 0.05) \); however, after 18 and 24 mo, the differences vanished. The mean relapse rate per patient increased to keep patients’ CsA trough levels in the defined target range of 80 to 150 ng/ml. The mean CsA concentrations were within this range; however, some individuals show low values of 35 ng/ml and others high levels of 355 ng/ml. Neither side effects nor efficacy of the CsA treatment could be correlated to individual dosing and trough levels. CsA absorption characteristics were not measured in this study.

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**Table 1. Demographic data of 104 patients who completed the initial treatment\(^a\)**

<table>
<thead>
<tr>
<th></th>
<th>Pred (n = 55)</th>
<th>Pred+CsA (n = 49)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>5.6 ± 3.2</td>
<td>5.1 ± 2.8</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>114 ± 19</td>
<td>109 ± 18</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>24.9 ± 13</td>
<td>21.9 ± 9.1</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>17.9 ± 2.7</td>
<td>17.8 ± 1.9</td>
</tr>
<tr>
<td>First symptoms until start of therapy (d)</td>
<td>7 (1 to 52)</td>
<td>10 (1 to 54)</td>
</tr>
</tbody>
</table>

\(^a\)Data are expressed as mean ± SD or mean (range). BMI, body mass index; Pred, 6 wk of 60 mg/m\(^2\) per d prednisone followed by 6 wk of 40 mg/m\(^2\) per 48 h; Pred+CsA, same prednisone treatment plus 8 wk of cyclosporine.

**Table 2. CsA blood levels and doses in 49 patients with NS during the 8-wk treatment period\(^a\)**

<table>
<thead>
<tr>
<th>Day</th>
<th>CsA Blood Levels (ng/ml)</th>
<th>CsA Dose (mg/m(^2))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>3</td>
<td>149</td>
<td>14</td>
</tr>
<tr>
<td>7</td>
<td>152</td>
<td>33</td>
</tr>
<tr>
<td>14</td>
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<td>41</td>
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<td>28</td>
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<td>35</td>
</tr>
<tr>
<td>42</td>
<td>152</td>
<td>38</td>
</tr>
<tr>
<td>52</td>
<td>160</td>
<td>82</td>
</tr>
</tbody>
</table>

\(^a\)According to the protocol, the CsA dose should be adjusted by the investigators to a target trough blood level range of 80 to 150 ng/ml. NS, nephrotic syndrome.
Safety and Tolerability

CsA treatment generally was well tolerated. Infections that were related to the combined prednisone plus CsA treatment were not reported. Major concerns of CsA treatment were nephrotic side effects, but kidney function did not show any deterioration. As shown in Figure 3, median serum creatinine concentrations as well as the range were identical in both groups at the end of 12 wk of initial treatment. In all patients, the GFR (calculated according to the formula of Schwartz [18]) was within the normal range. At the end of the study, serum creatinine was equal in both groups (46.2 ± 10.0 μmol/L in the Pred group and 48.2 ± 11.1 μmol/L in the Pred+CsA group).

BP increased transiently under CsA treatment, the mean systolic by 10 and diastolic by 8 mmHg, but normalized completely at the end of initial treatment (Figure 4). No patient received antihypertensive treatment.

Other expected CsA-associated side effects, such as hypertrichosis and gum hypertrophy, were seen more frequently under CsA treatment but resolved completely in all patients (Figure 5). Obesity was more often reported during the prolonged initial treatment with prednisone but was not significantly different among the groups. During the entire study period, striae distensae were encountered in 25.6% of patients of the Pred group and in 22.4% of patients of the Pred+CsA group. Psychologic disturbances were reported in 27% of CsA-treated patients compared with 14% in those who were treated with prednisone alone.

Risk Factors for Relapses

Despite some improvement in relapse rate by increasing the overall initial immunosuppression, the question remains as to why half of the patients experience relapses and half of them do not. To identify risk factors for relapses, we performed a multiple regression analysis and a logistic regression analysis. Demographic data such as age, height, weight, and gender and laboratory values at time of presentation before treatment as well as time to remission and CsA concentrations were entered.

Statistical analysis revealed that only age and protein concentration at initial manifestation were significant risk factors. Children who were younger than 7 yr and had an initial protein concentration of <44 g/L exhibited a higher risk for relapses and showed a significantly better sustained remission rate with initial CsA treatment (P = 0.03) for the 2-yr observation time (Figure 6).

As shown in Figure 7, logistic regression revealed that the age at time of initial manifestation correlates significantly with the risk for relapses after initial therapy. This effect is more pronounced in the Pred group compared with the Pred+CsA group. The risk for experiencing a relapse in 2- to 3-yr-old patients is 4 times higher than the risk for those who are older than 7 yr.

Discussion

The empirical introduction of corticosteroids for the treatment of minimal-change NS (MCNS) in the middle of the last century has improved morbidity and mortality. However, frequent relapses in approximately 40% of prednisone responders (19) led to steroid dependency and to severe steroid-induced side effects (5,20–23). Therefore, multicenter trials have been carried out to find treatment modalities that reduce the number of relapses and the rate of those who are steroid dependent and to reduce the glucocorticosteroid-induced side effects.

On the basis of a randomized, controlled trial, APN therefore proposed a so-called new standard initial therapy, which consisted of a 6-wk continuous prednisolone treatment followed by 6-wk alternate-day prednisone (2). The relatively high rate of steroid side effects was counterbalanced by a lower cumulative dose of steroids needed to treat relapses. For avoiding
higher doses of steroids, an add-on immunosuppressive drug, which has been shown to be effective in preventing relapses, seemed reasonable. Cyclophosphamide treatment might be an option, but side effects do not justify treating all patients at initial presentation, because approximately 50% will receive an unnecessary potentially harmful therapy.

Compared with other drugs, CsA has the advantage that its drug concentrations can be measured. This allows for the estimating of compliance as well as for the adjusting of the dose to a certain blood concentration, which has been shown to have immunosuppressive effects in other conditions, particularly in organ transplantation. It was clear, however, that the defined dose and trough level range for treatment of MCNS was based more on clinical experience (24) than on dose-response trials.

Up to now, dose-response studies were not available. From studies that used CsA in steroid-dependent NS, there is clear evidence that CsA is effective in preventing relapses for as long as the drug is given; however, after stopping, up to 90% of patients will develop a relapse immediately or at least within 90 d (25).

In this study, it is impressive that in the Pred+CsA group, patients had significantly fewer relapses for 1 yr after ending the initial therapy. Thereafter, the effect is attenuated, and after 2 yr, the relapse rate is almost the same in both groups. Many other data on outcomes, such as the mean number of relapses per patient (0.12 versus 0.57 after 6 mo, 0.63 versus 1.03 after 1 yr, and 1.03 versus 2.06 after 2 yr), the cumulative dose of steroids needed to treat relapses, and the number of patients who were treated with cyclophosphamide because they fulfilled the definition of steroid dependency, were lower in the Pred+CsA group (five versus 12), but a level of statistically significant difference was not demonstrable ($P = 0.1$). Combining all single factors may point toward a beneficial effect of the 8-wk add-on of CsA.

One may argue that the study was not powered sufficiently to test the hypothesis of a difference in the order of 10 to 20%. We did not aim to increase the number of patients for two reasons: First in the initial design, we planned to recruit approximately 150 patients, and, second, the biologic benefit of these differences seemed to be too low to justify this combined treatment even with statistical confirmation from a higher powered study. Concerning side effects, impairment of GFR has been reported after long-term CsA treatment, but also short-term treatment may lead to functional changes. Hulton et al. (26) reported a drop in GFR after 3 mo of treatment from 118 to 93 ml/min per 1.73 m², which was fully reversible after cessa-
tion of CsA. Despite this fact, histologic CsA-associated toxicity has been reported. The 8-wk CsA treatment in our study did not cause a measurable deterioration in GFR. However, there was a moderate effect on BP. This was transient and has not led to treatment with antihypertensive drugs in a single case.

The cosmetic side effects, such as hypertrichosis and especially gum hypertrophy, are more relevant and, fortunately, were completely reversible. This might be acceptable if the benefit in terms of preventing further relapses had been much clearer. Psychologic disturbances, although not specified further, mainly were disturbances of behavior and were of major concern. The necessity of a tight control of CsA blood levels with more frequent visits compared with the Pred group also was judged as a major disadvantage of the combined PredCsA treatment.

One goal of the study was to reduce the number of patients who meet the APN criteria for cyclophosphamide treatment. There was a short-term advantage, but the evolution toward the same 2-yr cumulative relapse rate points to a temporary effect only.

Recently, the Cochrane group (27) reviewed published trials on the impact of the length of prednisone therapy in children with a first episode of NS. A meta-analysis of six trials that compared 2 mo of prednisone with 3 mo or more for the first episode showed that a longer duration significantly reduced the risk for relapse at 12 to 24 mo (relative risk 0.70; 95% CI 0.58 to 0.84) without an increase in adverse events. There was an inverse linear relationship between duration of treatment and risk for relapse (relative risk 1.26 to 0.112 duration; \( R^2 = 0.56; \) \( P = 0.03 \)). They concluded that children in their first episode of steroid-sensitive NS should be treated for at least 3 mo, with an increase in benefit being demonstrated for up to 7 mo of treatment. These data support our hypothesis about the importance of the amount of initial immunosuppression but offers no alternative to the clinical burden of the high cumulative steroid therapy.

**Conclusion**

Eight weeks of add-on treatment with CsA at initial manifestation of steroid sensitive NS has an effect on the number of subsequent relapses that lasted for 1 yr. The attenuation of the effect after 2 yr, as well as the side effects and the need to control blood levels, discourage the recommendation of this protocol for all patients. The significantly higher risk for relapses of younger children is an important finding. Add-on CsA treatment seems to reduce the risk for younger children who experience relapses but does not abolish it. Further studies should stratify pediatric patients with NS according to these age groups, and intensified treatment should focus on the younger group.

Finally, it remains difficult to explain that the effect of CsA or the combination of steroids with CsA lasted much longer than the short-term effect of CsA on lymphocyte calcineurin inhibition and on inhibition of cytokine transcription and synthesis, which last for hours only. Considering MCNS as a disease of podocytes with a transient destabilization of their structure or function, it may be a reasonable hypothesis that CsA stabilizes podocyte structure or function by interfering with molecular podocyte targets rather than by an indirect immunosuppressive effect.

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