A Randomized Study Comparing Methylprednisolone Plus Chlorambucil Versus Methylprednisolone Plus Cyclophosphamide in Idiopathic Membranous Nephropathy

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Abstract. To assess whether chlorambucil or cyclophosphamide may have a better therapeutic index in patients with idiopathic membranous nephropathy, we compared two regimens based on a 6-mo treatment, alternating every other month methylprednisolone with chlorambucil or methylprednisolone with cyclophosphamide. Patients with biopsy-proven membranous nephropathy and with a nephrotic syndrome were randomized to be given methylprednisolone (1 g intravenously for 3 consecutive days followed by oral methylprednisolone, 0.4 mg/kg per d for 27 d) alternated every other month either with chlorambucil (0.2 mg/kg per d for 30 d) or cyclophosphamide (2.5 mg/kg per d for 30 d). The whole treatment lasted 6 mo: 3 mo with corticosteroids and 3 mo with one cytotoxic drug. Among 87 patients followed for at least 1 yr, 36 of 44 (82%; 95% confidence interval [CI], 67.3 to 91.8%) assigned to methylprednisolone and chlorambucil entered complete or partial remission of the nephrotic syndrome, versus 40 of 43 (93%; 95% CI, 80.9 to 98.5%) assigned to methylprednisolone and cyclophosphamide (P = 0.116). Of patients who attained remission of the nephrotic syndrome, 11 of 36 in the chlorambucil group (30.5%) and 10 of 40 in the cyclophosphamide group (25%) had a relapse of the nephrotic syndrome between 6 and 30 mo. The reciprocal of plasma creatinine improved in the cohort groups followed for 1 yr for both treatment groups (P < 0.01) and remained unchanged when compared with basal values in the cohort groups followed for 2 and 3 yr. Six patients in the chlorambucil group and two in the cyclophosphamide group did not complete the treatment because of side effects. Four patients in the chlorambucil group but none in the cyclophosphamide group suffered from herpes zoster. One patient per group developed cancer. It is concluded that in nephrotic patients with idiopathic membranous nephropathy both treatments may be effective in favoring remission and in preserving renal function for at least 3 yr. (J Am Soc Nephrol 9: 444–450, 1998)

There is now controlled evidence that a 6-mo course with methylprednisolone and chlorambucil may favor remission of the nephrotic syndrome (1–3) and may significantly improve the 10-yr kidney survival rate in patients with idiopathic membranous nephropathy (4). Randomized (5) and nonrandomized (6,7) trials showed that this regimen can improve kidney function and reduce proteinuria also in patients with established renal insufficiency.

Only a few nephrologists, however, are familiar with chlorambucil; the majority have experience with cyclophosphamide. Although cyclophosphamide and chlorambucil both have the capacity to contribute alkyl groups to biologically
vital macromolecules such as DNA, there are differences between the two drugs in dosing, metabolism, pharmacokinetics, and elimination. Thus, the results of treatment with either chlorambucil or cyclophosphamide might be different.

To evaluate whether these alkylating agents had similar effects when alternated with corticosteroids in idiopathic membranous nephropathy, we organized a multicenter, controlled trial. Patients with biopsy-proven membranous nephropathy and nephritic syndrome were randomly assigned to receive a 6-mo regimen with methylprednisolone alternated with chlorambucil or with the same doses of methylprednisolone alternated with cyclophosphamide.

Materials and Methods

Patients

Patients of either sex, between the ages of 14 and 65 with a biopsy-proven membranous nephropathy and a nephritic syndrome (defined as proteinuria exceeding 3.5 g/d and plasma albumin concentrations of <25 g/L), were considered for the study. The criteria for exclusion were a plasma creatinine >1.7 mg per deciliter (150 μmol/L); previous treatments with corticosteroids, immunosuppressive drugs, or cyclosporine; a positive serum test for anti-DNA antibodies (and antinuclear antibodies in many patients) or hepatitis B antigen or hepatitis C virus antibodies; a positive Veneral Disease Research Laboratory test; or low serum concentrations of the complement component C3 or C4. A clinical diagnosis of diabetes mellitus, malignancy, systemic lupus erythematosus, infections, or exposure to drugs that could induce membranous nephropathy were also criteria for exclusion.

Renal Histology

The pathologist at each study center examined each renal biopsy specimen by light, immunofluorescence, and electron microscopy. Glomerular stages were classified according to the system of Ehrenreich et al. (8). Mesangial glomerular sclerosis and interstitial fibrosis with tubular atrophy were classified as present or absent. No follow-up biopsies were performed.

Study Design

This was an open-label multicenter study. The protocol was approved by the institutional review board of the coordinating center, and each patient gave informed written consent. At the coordinating center, patients were assigned consecutively to one of the two treatment regimens, according to a center-stratified random order. A sample size of approximately 50 patients per group was necessary to demonstrate a decrease of about 25% of response from a baseline of 80% with methylprednisolone plus chlorambucil (2) at a power of about 0.80 with a two-tailed test (alpha = 0.05). Patients were examined at least every month during the first 6 mo, every 2 mo until the end of the first year, and every 3 to 6 mo thereafter. At each visit, plasma creatinine concentrations and 24-h urinary protein excretion rates were measured, and the patient was questioned about symptoms and possible side effects of therapy. Leukocyte, erythrocyte, and platelet counts were measured every 7 to 10 d during administration of the cytotoxic drug, and repeated at each visit.

In our previous experience with patients selected with the same criteria, only one of 39 untreated patients had a spontaneous remission of the nephritic syndrome within 6 mo (4). Therefore, we preferred to start early, without a run-in period, a potentially beneficial treatment to prevent complications of the nephrotic syndrome. Patients assigned to the chlorambucil group received three cycles of treatment with methylprednisolone (1 g given intravenously on 3 consecutive days and then 0.4 mg/kg body wt per d given orally for 27 d, in a single morning dose), and each cycle was followed by 1 mo of treatment with chlorambucil (0.2 mg/kg per d, orally). Patients assigned to the cyclophosphamide group received the same three cycles of methylprednisolone, but chlorambucil was replaced by oral cyclophosphamide at a dose of 2.5 mg/kg body wt per d. If a patient’s leukocyte count decreased below 5000 per cubic millimeter (5 × 10^9/L), the dose of the cytotoxic agent was reduced by 50%, and it was discontinued for the remainder of that cycle if the leukocyte count was less than 3000 per cubic millimeter (3 × 10^9/L). The total duration of treatment, therefore, was 6 mo for both groups; 3 mo with the same doses of methylprednisolone and 3 mo with either of the two cytotoxic drugs. Steroids were completely stopped at the end of the study period. The use of angiotensin-converting enzyme inhibitors was discouraged but not prohibited. Hypolipemic drugs were permitted.

Clinical response was defined as a complete or partial remission. Complete remission was defined as a reduction in the rate of urinary protein excretion to ≤0.2 g/d for at least 1 wk, and partial remission as a reduction in the rate of protein excretion to between 0.21 and 2 g/d for at least 1 wk, with a normal plasma creatinine concentration. Relapse of the nephrotic syndrome was defined as an increase in proteinuria to more than 3.5 g/d for at least 1 wk in patients who had a remission. Stable worsening was defined as an increase in plasma creatinine concentration of at least 50% over the baseline value.

Statistical Analyses

Cumulative probability of complete/partial remission, complete remission alone, and thereafter of relapses was estimated according to Kaplan and Meier (9). Because assumption of the hazard functions proportionality cannot be considered fulfilled as suggested by the log (−log) survival plots, survival curves have been shown for illustrative purposes only; χ² tests have been performed on the cumulative proportion of patients with complete or partial remission. A further index of efficacy was obtained as the percentage of the remission time (partial or complete) on the total follow-up for each patient. The two treatments were then compared by the Wilcoxon rank sum test.

The reciprocal of the plasma creatinine and proteinuria values for the two treatments were compared by mixed-factorial ANOVA for repeated measurements (10), with treatment as a fixed, “between-patient” factor at two levels and time as a fixed, “within-patient” factor at 21 levels (every 6 mo). The Geisser-Greenhouse (11) procedure for correction of the degrees of freedom for statistical significance of the within-patient terms was followed. In addition, a trend analysis has been carried out by means of orthogonal polynomial contrasts to assess the presence of a linear increasing/decreasing trend (first degree), of an increasing and then decreasing (or vice versa) trend (second degree), of an increasing-decreasing-increasing or decreasing-increasing-decreasing trend (third degree) of a mean fluctuating pattern (degree higher than the third). Indeed, the shape of an overall pattern could have therapeutic impact.

Results

Among 97 patients who satisfied the criteria for randomization, two were wrongly randomized due to the presence of underlying neoplasias (discovered, respectively, 1 wk and 2 mo after randomization). Of the remaining 95 patients, 50 were assigned to receive methylprednisolone and chlorambucil, whereas 45 were randomized to methylprednisolone and cyclophosphamide. However, two patients did not present at the
follow-up visit and a 51-yr-old woman died because of a deep-vein thrombosis with acute renal failure and cardiac shock 3 mo after the diagnosis of membranous nephropathy, before treatment was started. There was no difference at presentation between the two groups in the main clinical, biological, and histological features (Table 1).

Four patients in the chlorambucil group and one in the cyclophosphamide group, who completed the treatment, did not present at the follow-up visit and were considered lost to follow-up after the sixth month. All of the other patients were followed for at least 1 yr (range, 12 to 78 mo). The median period of follow-up was 36 mo (range, 12 to 78 mo) in the chlorambucil group and 42 mo (range, 12 to 72 mo) in the cyclophosphamide group, with a very similar distribution.

Of the 87 patients followed for at least 1 yr, 76 (87.3%; 95% CI, 78.5 to 93.5%) entered a complete (28 patients) or partial remission (48 patients). The presence or absence of interstitial fibrosis or mesangial sclerosis and the stage of glomerular lesions at renal biopsy did not influence the likelihood remission of nephrotic syndrome. However, if one takes into account the complete remission only this was significantly related to the presence (8.7%; 95% CI, 10.7 to 28.0%) or absence (40.6%; 95% CI, 28.5 to 53.6%) of mesangial glomerular sclerosis (P = 0.005). Of 44 patients assigned to methylprednisolone and chlorambucil, 36 (82%; 95% CI, 67.3 to 91.8%) entered complete (12 patients) or partial remission (24 patients) of proteinuria as a first event. Of the 43 patients given methylprednisolone and cyclophosphamide, 40 (93%; 95% CI, 80.9 to 98.5%) entered complete (16 patients) or partial remission (24 patients) as a first event. The difference between the two groups was not significant (P = 0.116). The curves of the probability of a partial or a complete remission after treatment were similar in the two study groups (Figure 1).

Eleven patients in the chlorambucil group and 10 in the cyclophosphamide group had a relapse of the nephrotic syndrome. All of the relapses occurred between the sixth and the thirtieth month (Figure 2). Four patients were retreated. In the chlorambucil group, two patients were retreated with steroids and chlorambucil. One did not respond, and the other attained partial remission. In the cyclophosphamide group, one patient was retreated with steroids and cyclophosphamide and had complete remission. Another patient was treated with steroids and chlorambucil and had partial remission. The median of the percentage of time spent without nephrotic syndrome was 60% of the whole period of observation for the entire study group, without significant difference between the group given chlorambucil (58.6%) and that given cyclophosphamide (62.5%, P = 0.534). We considered separately patients with complete follow-ups of 12, 24, and 36 mo, respectively, to have more precise estimates on a greater number of patients for the 1- and 2-yr analyses. For both of the treatment groups, there was a significant decrease in proteinuria when compared with the basal value for the patients with a complete follow-up of 12, 24, and 36 mo (P = 0.0001). Instead, differences between the two treatment groups turned out not to be statistically significant. After an early relevant decrease of proteinuria at 6 mo, there was a fluctuating pattern of approximately 2 g/d, similar in the two treatment groups (Figure 3) as confirmed by the significance (P < 0.0001) of the orthogonal polynomials higher than the third degree.

The reciprocal of plasma creatinine was significantly (at least P < 0.0055) higher at 6 and 12 mo for both treatment

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**Table 1. Characteristics of patients at the start of treatment with methylprednisolone plus chlorambucil or methylprednisolone plus cyclophosphamide**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>MP + Chlorambucil (n = 50)</th>
<th>MP + Cyclophosphamide (n = 45)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (median)</td>
<td>50</td>
<td>48</td>
</tr>
<tr>
<td>Range</td>
<td>18 to 65</td>
<td>17 to 55</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>37/13</td>
<td>29/16</td>
</tr>
<tr>
<td>Plasma creatinine (mg/dl)b</td>
<td>1.06 ± 0.27</td>
<td>1.04 ± 0.27</td>
</tr>
<tr>
<td>Urinary protein excretion (g/d)</td>
<td>7.96 ± 5.19</td>
<td>6.85 ± 3.51</td>
</tr>
<tr>
<td>Hypertensive patients (ACE inhibitors)</td>
<td>15 (1)</td>
<td>14 (2)</td>
</tr>
<tr>
<td>Stage of glomerular lesions at renal biopsy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I or II</td>
<td>32</td>
<td>27</td>
</tr>
<tr>
<td>III or IV</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>Mesangial sclerosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>yes</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td>no</td>
<td>36</td>
<td>33</td>
</tr>
<tr>
<td>Tubulointerstitial lesions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>yes</td>
<td>11</td>
<td>13</td>
</tr>
<tr>
<td>no</td>
<td>39</td>
<td>32</td>
</tr>
</tbody>
</table>

*Plus-minus values are means ± SD. Data for two patients (one per group) wrongly randomized are not included in this table. MP, methylprednisolone; ACE, angiotensin-converting enzyme.

b To convert values for creatinine to micromoles per liter, multiply by 88.4.
groups (without a statistically significant difference between them) when compared with baseline, followed by a mild decrease to values similar to baseline, at least for patients followed for 3 yr (Figure 4). One patient in the chlorambucil group had worsening of renal function and had to be submitted to regular dialysis after 42 mo of follow-up. Two patients in the cyclophosphamide group worsened, and one of them had to be submitted to regular dialysis after 36 mo of follow-up (Table 2).

Six patients (12%) did not complete treatment in the chlorambucil group. Two of them did not receive chlorambucil at the sixth month because of leukopenia, which reversed spontaneously. Two patients developed pneumonia after 5 and 3 mo of therapy, respectively. Both of them recovered but continued without chlorambucil. One patient developed anemia and thrombocytopenia with hypoplasia at the bone marrow aspiration after 1 mo of chlorambucil. He stopped treatment with chlorambucil and recovered. Another patient stopped chlorambucil treatment because of nausea after a few days. Two patients (4.5%) stopped treatment in the cyclophosphamide group, one because of nausea and vomiting after a few days. The other patient stopped treatment after 2 mo because of a cerebral transient ischemic attack. One patient per group developed glucose intolerance during treatment, which reversed after completion of treatment. Four patients in the chlorambucil group, but none in the cyclophosphamide group, developed herpes zoster during treatment. In the long-term, a 40-yr-old woman treated with chlorambucil had irreversible amenorrhea. In the chlorambucil group, a man 58 yr old developed a laryngeal carcinoma and underwent a laryngectomy after 48 mo. In the cyclophosphamide group, a 58-yr-old man was recognized to have a prostatic carcinoma 66 mo after randomization. Both patients are still alive after 12 and 6 mo, respectively.

**Discussion**

In this study, a 6-mo course with methylprednisolone and chlorambucil favored the remission of the nephrotic syndrome in patients with idiopathic membranous nephropathy. After treatment, the mean proteinuria significantly decreased compared with baseline at any time point considered. Eighty-two percent of patients given methylprednisolone and chlorambucil attained complete or partial remission of the nephrotic syndrome as a first event, a proportion similar to the 81% (2) and the 75% (3) found, respectively, in two previous different controlled studies of Italian patients selected with the same criteria and treated with methylprednisolone and chlorambucil for 6 mo. In the study presented here, some 30% of patients had a relapse of the nephrotic syndrome, which occurred between the sixth and the thirtieth month.

Similar results were observed in the group randomized to methylprednisolone and cyclophosphamide. The mean decrease in proteinuria was similar to that seen in patients given chlorambucil at any month cohort investigated. Ninety-three percent of patients entered remission of the nephrotic syndrome as a first event, and 24% had a relapse of the nephrotic syndrome between the sixth and the twenty-fourth month. However treated, patients spent 60% of the period of observation without nephrotic syndrome. The percentage of time in remission was similar in the two study groups and was almost the same as the 58% found in a previous trial with methylprednisolone and chlorambucil (4). These data show that either chlorambucil or cyclophosphamide alternated with methylprednisolone can result in a sustained remission of the nephrotic syndrome in most patients. This result may have a clinical impact if one considers that nephrotic patients are at a greater risk of coronary heart disease (12) and thrombotic events, particularly when they have membranous nephropathy.
as the underlying disease (13,14). Moreover, persistent proteinuria may make them susceptible to impaired renal function in the long-term (15,16).

The second outcome measure of this trial was the effect of treatment on renal function, as assessed by the reciprocal of plasma creatinine over time. In both groups, the mean reciprocal of plasma creatinine improved in the 12-mo cohort and remained stable in either group in the 24- and 36-mo cohorts.

The follow-up period of this study was short, but it has been shown that following the reciprocal of plasma creatinine even for a short time may predict the long-term prognosis in patients with membranous nephropathy (17). Although these data should be extrapolated with caution, one may speculate that these patients should have little risk of developing renal insufficiency even in the long-term. This view is reinforced by previous results showing that patients who attained a complete remission (18) or even partial remission (19) of the nephrotic syndrome were unlikely to develop any deterioration of renal function in the long-term follow-up.

The use of corticosteroids and alkylating agents may expose these patients to side effects. Several patients had to stop treatment because of adverse effects. In all cases, patients with side effects that developed during treatment recovered completely after stopping treatment and adequate management. The rate of adverse events leading to discontinuation in patients given chlorambucil was 12%, similar to the 9.5% (2) and 9% (3) that we observed in two previous controlled trials with methylprednisolone and chlorambucil. A smaller number of patients given cyclophosphamide had to stop treatment because of side effects. Moreover, none of them suffered from herpes.

#### Table 2. Clinical status at the last follow-up visit

<table>
<thead>
<tr>
<th>Status</th>
<th>Chlorambucil (44 patients)</th>
<th>Cyclophosphamide (43 patients)</th>
</tr>
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<tbody>
<tr>
<td>Follow-up (median)</td>
<td>36</td>
<td>42</td>
</tr>
<tr>
<td>Complete remission</td>
<td>12 (27.2%)</td>
<td>16 (37.2%)</td>
</tr>
<tr>
<td>Partial remission</td>
<td>24 (54.5%)</td>
<td>24 (55.8%)</td>
</tr>
<tr>
<td>Stable</td>
<td>7 (15.9%)</td>
<td>1 (2.3%)</td>
</tr>
<tr>
<td>Worsened</td>
<td>1 (2.3%)</td>
<td>2 (4.6%)</td>
</tr>
<tr>
<td>Mean proteinuria</td>
<td>2.11 ± 2.89</td>
<td>1.69 ± 2.36</td>
</tr>
<tr>
<td>(g/dl)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean plasma creatinine (mg/dl)</td>
<td>1.25 ± 1.37</td>
<td>1.32 ± 1.72</td>
</tr>
</tbody>
</table>
zoster, which occurred in four patients given chlorambucil. The main concern with the use of cytotoxic drugs is the potential risk of neoplasia in the long-term. Leukemia and, more rarely, lymphoma (20,21) are the most frequent types of cancer that develop in patients given chlorambucil, whereas bladder cancer and lymphoreticular tumors (22,23) are the most frequent malignancies in patients treated with cyclophosphamide. None of our patients suffered from any of these tumors. It is possible, however, that alkylating agents may expose them to other forms of neoplasia. In immuno suppressed patients, the hazard of neoplasia is roughly related to the cumulative dose and to the duration of cytotoxic therapy (24–26). Whether short-term administration of an alkylating agent increases the risk of cancer in patients with membranous nephropathy is still unclear. The problem is further complicated by the fact that membranous nephropathy may be the first clue of an underlying cancer (27,28). Even in this study, two patients were recognized to have malignancy only after randomization. One patient received steroids for only a few days, whereas the other patient did not receive any treatment for membranous nephropathy. On the other hand, we observed one case of prostatic cancer 5 yr after treatment in the group given chlorambucil and one case of laryngeal cancer 4 yr after treatment in the group given cyclophosphamide. To evaluate whether these cancers represented a chance event or a complication of treatment, we compared the cumulative risk of cancer in patients given methylneprosolone and chlorambucil for 6 mo with that of a general white population. To increase the number of patients at risk (in addition to the patients of this study), 79 other patients treated with steroids and chlorambucil who participated to two previous controlled trials in membranous nephropathy (3.4) were considered. Therefore, we were able to collect information about 662 patients/years treated with methylneprosolone and chlorambucil for 6 mo. In this population, we observed three cases of cancer: one in the present study and two cases in the two previous controlled studies. Thus, the cumulative risk of developing cancer in our patients given methylneprosolone and chlorambucil was 4.53/1000 patients per year (95% CI, 0.935 to 13.241). This hazard is similar to that observed in a general white population, being the average annual incidence of primary cancer among white population (4.33/1000 for men and 3.40/1000 for women) (29). We do not have controlled data with the use of cyclophosphamide other than those reported in this trial, clearly too a small number of patients to draw any conclusion.

In conclusion, this controlled trial confirms that the alternation of steroids and cytotoxic drugs for 6 mo favors remission of the nephrotic syndrome and protects from renal dysfunction. No significant difference could be observed between patients given chlorambucil or cyclophosphamide. On the basis of these results, either of these cytotoxic drugs may be used in membranous nephropathy whenever the clinician feels that a particular patient should be treated.

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References


