Treatmen-related changes in urinary excretion of high and low molecular weight proteins in patients with idiopathic membranous nephropathy and renal insufficiency

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Abstract

Background. In patients with idiopathic membranous nephropathy, an increased urinary excretion of high (IgG) and low [β2-microglobulin (β2M), α1-microglobulin (α1M)] molecular weight proteins predicts prognosis and precedes renal insufficiency. We have studied the changes in the urinary excretion of these proteins in patients with idiopathic membranous nephropathy and renal insufficiency during and after treatment with cyclophosphamide and steroids, and investigated their value in predicting long-term outcome.

Methods. Standardized measurements of urinary IgG, albumin, β2M and α1M were performed at 0, 2, 6 and 12 months in 11 patients, at 12 months in 25 patients and in 17 of these last patients after 2–5 years.

Results. We observed a rapid improvement of glomerular permselectivity and tubular protein reabsorption within 2 months after the start of therapy. Despite a partial remission of proteinuria within 12 months in most patients, evidence of tubulo-interstitial injury remained apparent. Neither absolute levels of urinary IgG, β2M or α1M at baseline or at 12 months nor the percentage reduction between baseline and 12 months clearly predicted the occurrence of a remission or a relapse to nephrotic range proteinuria. In the case of a persistent stable remission, we observed a gradual decrease of urinary β2M towards normal values.

Conclusions. In patients with idiopathic membranous nephropathy and renal insufficiency, treatment with cyclophosphamide and steroids resulted in an improvement of glomerular permeability and tubular proteinuria. Tubular proteinuria remained present for many years, even in patients with stable remission of proteinuria. Measurements of urinary proteins at 12 months after treatment start lacked predictive accuracy.

Keywords: immunosuppressive therapy; membranous nephropathy; renal insufficiency; urinary α1-microglobulin; urinary β2-microglobulin; urinary IgG

Introduction

Idiopathic membranous nephropathy is the most common cause of the nephrotic syndrome in adults [1]. Approximately 40% of patients with idiopathic membranous nephropathy and a nephrotic syndrome will progress to renal insufficiency [2–4]. We and others have demonstrated that the urinary excretion of the high molecular weight protein IgG and of the low molecular weight proteins β2-microglobulin (β2M) and α1-microglobulin (α1M) accurately predicts prognosis in patients with idiopathic membranous nephropathy and normal renal function [5–8]. Severe alterations in glomerular permselectivity (identified by non-selective proteinuria [9] and high levels of urinary IgG) are associated with tubulo-interstitial injury (identified by high levels of urinary β2M and α1M), which ultimately causes renal insufficiency [10–12]. In our study [7] in multivariate analysis, urinary β2M excretion proved the strongest independent predictor for the development of renal failure, which is in agreement with the observations that (development of) renal insufficiency correlates better with tubulo-interstitial damage than with glomerular injury [11].

We recently have reported that immunosuppressive therapy consisting of cyclophosphamide and steroids is effective in patients with idiopathic membranous nephropathy and renal insufficiency. In most patients, renal function improved, and >80% of patients...
developed a partial remission of proteinuria. Unfortunately, relapses occurred in 28% of patients after 5 years follow-up [13,14].

Over the last years, we have quantitated urinary high and low molecular weight proteins during and after treatment. We have analysed the data, specifically evaluating the response of glomerular permselectivity characteristics and tubulo-interstitial injury in time. We also questioned if measurement of these proteins at the end of the treatment year allows the prediction of prognosis.

Subjects and methods

We recently evaluated the efficacy of treatment with oral cyclophosphamide and steroids in patients with idiopathic membranous nephropathy, nephrotic syndrome and renal insufficiency [14]. Treatment consisted of oral cyclophosphamide in a dose of 1.5–2.0 mg/kg body weight/day for 12 months, together with steroids. The corticosteroid regimen consisted of three consecutive intravenous (i.v.) pulses of 1 g of methylprednisolone at months 0, 2 and 4, and oral prednisone, in a dose of 0.5 mg/kg body weight, on alternate days for 6 months. In patients treated most recently, standardized measurements of urinary proteins and renal function were performed at the indicated time intervals after start of therapy. Twenty-five patients were studied at the end of treatment (12 months). In addition, the time course of changes in proteinuria was studied more closely in 11 patients who were evaluated at 0, 2, 6 and 12 months. Measurements were repeated after longer follow-up in 17 patients.

Standardized measurement of urinary proteins and renal function

All patients collected two 24 h urine samples for measurement of creatinine, total protein, urea and sodium. The excretion of the low and high molecular weight proteins was measured under standardized conditions. In brief, patients came to the ward after an overnight fast. They received 4000 mg of oral sodium bicarbonate the evening before, and additionally given to inhibit tubular secretion of creatinine, but has been shown not to influence the glomerular permeability and tubular reabsorption of proteins [15]. On arrival at the ward, patients had taken 1200 mg of cimetidine orally. Cimetidine was given to inhibit tubular secretion of creatinine, but has been shown not to influence the glomerular permeability and tubular reabsorption of proteins [15]. On arrival at the ward, patients had taken 1200 mg of cimetidine orally. 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Blood pressure measurements were taken using an automated device (DINAMAP, Criticon, Tampa FL), with six consecutive readings registered every 5 min after 10 min rest; these readings were used to calculate the average mean arterial pressure (MAP). The timed urine sample, collected after 2 h, was used for the measurement of urinary pH, β2M, α2M, IgG, transferrin, albumin, total protein and creatinine. Only in urine with a urinary pH >6.0 was β2M excretion measured. Laboratory parameters were measured in blood samples collected in the middle of the urine collection period.

The use of angiotensin-converting enzyme inhibitors (ACEIs) and/or angiotensin II type 1 receptor antagonists (ARBs), calcium channel blockers, non-steroidal anti-inflammatory drugs (NSAIDs) and spironolactone as well as HMG-CoA-reductase inhibitors (statins) was noted.

Laboratory measurements

Serum creatinine, cholesterol, urinary total protein, creatinine, urea and sodium were measured with standard automated techniques. The concentrations of α2M, albumin, transferrin and IgG in serum and urine were measured by immunonephelometry on a BNII nephelometer (Behring, Marburg, Germany) using antibodies whose specificity was checked by Ouchterlony double immunodiffusion and immunoelectrophoresis (Dako, Glostrup, Denmark). Serum and urinary β2M were measured by enzyme-linked immunosorbent assay (ELISA), as described before [16].

Calculations and statistics

Creatinine clearance was calculated according to the formula: Ucr × V/Per, where Ucr and Per are the concentration of creatinine in urine and plasma, respectively, and V is the urine flow.

Proteinuria (urinary total protein) is expressed as g/10 mmol creatinine. Use of this index does not affect the conclusions since the protein/creatinine index is highly correlated with proteinuria in g/day in 24 h urine collections (Pearson correlation 0.912, P <0.01). The excretion of the low and high molecular weight proteins is expressed per unit of time (minutes or 24 h) to allow comparison with our previously reported threshold values: for U1G 250 mg/24 h [5,7], for Uβ2M 500 ng/min (=0.5 μg/min) [6,7] and for α2M 40 μg/min [7,8]. Dietary protein and salt intake were deduced from the amount of urea and sodium excreted in 24 h urine.

The selectivity index (SI) of proteinuria was calculated using the formula: SI = (U1G/S1G) × (STransf/UTransf), where U = urine, S = serum, Transf = transferrin. Non-selective proteinuria was defined as an SI ≥ 0.21.

Although not the purpose of this study, the data have allowed us to study the relationship between the urinary excretion of IgG and the tubular reabsorption of β2M. Based on animal experiments, it has been suggested that an increased urinary excretion of β2M could result from competitive inhibition of tubular protein reabsorption. Previously we have provided evidence that albumin does not interfere with the tubular reabsorption of low molecular weight proteins [17]; however, in that study, we could not exclude an effect of IgG. For this purpose, the tubular reabsorption of β2M was calculated using the formula: reabsorption = 1 – fractional excretion (FE), and expressed as a percentage. FE of β2M = (Uβ2M/Sβ2M)/(Ucr × 1000)/Scr.

Values are given as medians with range. The Wilcoxon signed rank test was used for comparison of paired data on different time points. The Mann-Whitney U-test was used for comparison of data between different groups of subjects. A P-value <0.05 was considered significant. All statistical procedures were done using SPSS software (SPSS version 11.5, Chicago, IL).
Definitions

A complete remission of proteinuria, partial remission, persistent proteinuria and nephrotic range proteinuria were defined as a protein-creatinine index of <0.2, 0.21–2.0, 2.1–3.4 and ≥3.5 g/10 mmol creatinine, respectively, where in the case of remission renal function should have improved or at least stabilized. Relapses of proteinuria were defined as nephrotic range proteinuria after a partial or complete remission of the proteinuria or a rise in proteinuria of >50% in patients in whom proteinuria had improved initially with >50%, without reaching values ≥2.0 g/10 mmol creatinine.

Results

Time course of changes in glomerular permselectivity and tubular proteinuria during treatment (Tables 1–4)

Eleven patients were studied at the indicated time points (0, 2, 6 and 12 months) during the treatment year. Patients were all male, with a median age of 61 (45–75) years. The time between the diagnostic renal biopsy and the baseline study of proteinuria at the start of immunosuppressive therapy was 14 (2–126) months. We did not perform repeated biopsies in patients with deteriorating renal function.
Table 2. Serum measurements and calculated creatinine clearances in 11 patients with measurements at all time points (0, 2, 6 and 12 months) during the treatment year

<table>
<thead>
<tr>
<th>t = 0 months</th>
<th>t = 2 months</th>
<th>t = 6 months</th>
<th>t = 12 months</th>
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</thead>
<tbody>
<tr>
<td><strong>Serum values</strong></td>
<td><strong>Calculated creatinine clearances</strong></td>
<td><strong>Serum values</strong></td>
<td><strong>Calculated creatinine clearances</strong></td>
</tr>
<tr>
<td><strong>S creatinine (µmol/l)</strong></td>
<td>152 (132–278)</td>
<td>137 (108–221)</td>
<td>126 (83–168)</td>
</tr>
<tr>
<td><strong>S β₂M (mg/l)</strong></td>
<td>5.32 (3.13–7.52)</td>
<td>3.45 b (2.38–5.72)</td>
<td>2.80 b (2.20–4.60)</td>
</tr>
<tr>
<td><strong>S albumin (g/l)</strong></td>
<td>26 (18–35)</td>
<td>30 b (20–38)</td>
<td>36 b (32–39)</td>
</tr>
<tr>
<td><strong>S IgG (g/l)</strong></td>
<td>4.6 (2.4–13.2)</td>
<td>2.6 b (1.0–4.8)</td>
<td>3.6 b (2.1–10.6)</td>
</tr>
<tr>
<td><strong>S IgG (g/l)</strong></td>
<td>6.7 (4.8–18.0)</td>
<td>6.4 (4.1–10.0)</td>
<td>5.3 a (4.0–6.3)</td>
</tr>
<tr>
<td><strong>24 h urine (ml/min)</strong></td>
<td>53 (25–68)</td>
<td>59 b (29–100)</td>
<td>68 b (45–108)</td>
</tr>
<tr>
<td><strong>2 h urine (ml/min)</strong></td>
<td>39 (5–52)</td>
<td>52 b (21–68)</td>
<td>50 b (33–81)</td>
</tr>
</tbody>
</table>

*P < 0.05; bP < 0.01 of values compared with baseline values. S = serum; β₂M = β₂-microglobulin.

Table 3. Urine measurements in 11 patients with measurements at all time points (0, 2, 6 and 12 months) during the treatment year

<table>
<thead>
<tr>
<th>t = 0 months</th>
<th>t = 2 months</th>
<th>t = 6 months</th>
<th>t = 12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Proteinuria (g/10 mmol creatinine)</strong></td>
<td>11.7 (6.3–31.3)</td>
<td>5.0 b (1.4–9.4)</td>
<td>1.7 b (0.7–5.9)</td>
</tr>
<tr>
<td><strong>Albuminuria (g/10 mmol creatinine)</strong></td>
<td>7.9 (4.9–16.6)</td>
<td>3.4 a (0.9–6.4)</td>
<td>1.2 a (0.5–3.8)</td>
</tr>
<tr>
<td><strong>UE β₂M (µg/min)</strong></td>
<td>15.1 (2.5–46.9)</td>
<td>3.2 (0.3–56.8)</td>
<td>1.8 (0.2–37.6)</td>
</tr>
<tr>
<td><strong>UE S albumin (g/l)</strong></td>
<td>69.0 (17.0–306.7)</td>
<td>55.1 (17.0–182.2)</td>
<td>30.4 (7.3–126.1)</td>
</tr>
<tr>
<td><strong>UE S IgG (mg/24 h)</strong></td>
<td>348 (208–187)</td>
<td>61 b (25–283)</td>
<td>16 b (4–105)</td>
</tr>
<tr>
<td><strong>Fractional excretion of IgG</strong></td>
<td>0.22 (0.08–0.43)</td>
<td>0.03 a (0.00–0.24)</td>
<td>0.006 b (0.00–0.08)</td>
</tr>
<tr>
<td><strong>S creatinine (m mol/l)</strong></td>
<td>192 (132–278)</td>
<td>137 (108–221)</td>
<td>126 (83–168)</td>
</tr>
<tr>
<td><strong>S cholesterol (mmol/l)</strong></td>
<td>6.7 (4.8–18.0)</td>
<td>6.4 (4.1–10.0)</td>
<td>5.3 a (4.0–6.3)</td>
</tr>
</tbody>
</table>

*P < 0.05; bP < 0.01 of values compared with baseline values. S = serum; β₂M = β₂-microglobulin.

Detailed information on the use of the relevant medication is given in Table 1. All patients used an ACEI and/or an ARB at the start and during the treatment year, and seven patients used a statin for most of the treatment year. No patient used NSAIDs. No other immunosuppressive drugs were allowed other than those under study.

From the 24 h urine collections, we calculated a mean protein intake of 0.81 ± 0.25 g/kg body weight/day, indicating that the patients adhered to their advised protein-restricted diet. Mean salt intake was calculated at 7.7 ± 2.9 g/day, higher than advised. Mean arterial pressure was 90 (69–112) mmHg at baseline and did not change significantly during the treatment year.

Data on laboratory results are given in Tables 2–4. All patients had renal insufficiency as reflected by serum creatinine and calculated creatinine clearances. Creatinine clearances calculated from the 2 h urine, collected after cimetidine, are significantly lower than creatinine clearances calculated from 24 h urine, reflecting the inhibition of creatinine secretion by cimetidine. All patients had nephrotic range proteinuria, ranging from 6.3 to 31.3 g/10 mmol creatinine. Renal function improved in all patients. We also observed a reduction of proteinuria in all patients. All but one patient developed a partial remission, but no patient developed a complete remission within the first year of treatment. The decrease of proteinuria was paralleled by a significant rise in serum albumin and decrease of serum cholesterol. From Table 2, it is evident that serum IgG did not rise in parallel with serum albumin. Compared with baseline, lower values of serum IgG were observed at 2 and 6 months after the start of therapy. As expected, the urinary excretion of albumin decreased roughly in parallel with the decrease in urinary total protein. Urinary excretion of IgG decreased to a greater extent.

Detailed information on the changes in the urinary excretion of the various low and high molecular weight proteins per patient can be derived from Table 4. The time course of the urinary excretion of β₂M could only be fully assessed in seven patients in whom the urinary pH was above 6.0 in all measurements. Still, it is evident from Tables 3 and 4 that we observed a rapid and large reduction in urinary excretion of β₂M. A similar pattern was observed for urinary γ₁M, which was measurable in all patients. Although all patients but one attained a partial remission of proteinuria at 12 months, urinary excretion of the low molecular weight proteins remained abnormal in all but one patient at this time point.

Due to the high efficacy of treatment, evaluation of the value of the urine markers at the start of therapy to predict a remission of proteinuria was meaningless.
Predictive value of tubular proteinuria at the end of the treatment year (Table 5)

Twenty-five patients [21 males, four females; age 58 (38–75) years] were studied at the end of the treatment year (12 months); these include the 11 patients who were studied in more detail during the treatment year. Follow-up from the start of treatment lasted 36 (14–76) months.

Detailed information on the use of relevant co-medication is given in Table 1. All but two patients used an ACEI and/or ARB, and 19 patients used a statin. NSAIDs were not used.

During follow-up, 22 patients (88%) developed a partial remission of proteinuria after 7 (1–42) months; in 19 of them the partial remission was evident within the treatment year. Four of these 22 patients have improved to complete remission after 18 (9–22) months after the start of the treatment. In two patients, proteinuria improved to values <3.5 g/10 mmol creatinine (persistent proteinuria), whereas one patient had a persistent nephrotic syndrome. During follow-up, five patients relapsed to nephrotic range proteinuria (one from a complete remission, three from a partial remission and one from persistent proteinuria), after 34 (23–45) months. Thus, at the end of follow-up, 18 patients were in remission (three in complete remission, 15 in partial remission), one had persistent proteinuria and six patients had a (relapse to) nephrotic syndrome.

Renal function had improved in all patients, from a serum creatinine of 161 (112–444) mmol/l at the start of treatment to the lowest value of 114 (70–255) mmol/l (P<0.01) after a median of 10 months. Serum creatinine amounted to 120 (74–255) mmol/l at 12 months and 122 (79–267) mmol/l (NS) at the end of follow-up. A significant deterioration of renal function (defined as a 50% increase of serum creatinine over the lowest value obtained after treatment start) had occurred in two patients during follow-up; both patients also had a relapse of proteinuria to a nephrotic syndrome. Thus far, no patient has developed end-stage renal disease.

Values of serum and urine parameters for the 25 patients at the end of the 12 month treatment period are given in Table 5. At this time point, urinary IgG excretion had decreased to values below the threshold value of 250 mg/24 h in all patients. In contrast, the urinary excretion of β₂M and α₁M reached values below the thresholds in eight out of 21 and 16 out of 25 patients, respectively.
We next evaluated if the urinary excretion of $\beta_2$M, $\alpha_1$M and IgG at 12 months was a useful predictor of outcome. Since only two patients had evidence of renal failure during follow-up, we have defined failure as the presence of a nephrotic syndrome at the end of follow-up ($n=6$). Time to failure was either the total follow-up time in cases where no failure occurred, or time to relapse in the case of a relapse to nephrotic syndrome or start of repeated immunosuppressive therapy in the one patient with a persistent nephrotic syndrome. For univariate analysis, we have made Kaplan–Meier curves comparing patients with levels of the parameter under study above or below the median. No significant differences were found for the following parameters: urinary excretion of IgG, $\beta_2$M and $\alpha_1$M, serum creatinine and creatinine clearance, and serum albumin. Also, the percentage change in the urinary excretion of IgG, $\beta_2$M and $\alpha_1$M from the start till the end of immunosuppressive therapy had no predictive value. Thus, the measurements at 12 months do not allow the prediction of treatment failure or relapse.

In a subgroup analysis, we have investigated the possibility of predicting failure defined as a relapse of proteinuria after an initial remission. We only considered those patients ($n=12$) with a partial remission of proteinuria [at 7 (1–15) months], a follow-up time of $>24$ months after the occurrence of the remission, and with a standardized urine measurement at baseline and 12 months. Three patients failed. Again, the values at 12 months did not predict the development of a relapse. Also, the percentage improvement from baseline to 12 months was not predictive. All patients with a relapse had baseline values above the median for the total group. Although this suggested that baseline values could offer some clues about outcome after therapy, we could not confirm this in a larger group of treated patients who had baseline measurements at the start of therapy ($n=24$).

**Progressive improvement in tubular proteinuria in the case of a stable remission**

Since urinary $\beta_2$M excretion was still abnormal in most patients at 12 months, we have continued to study patients during a longer follow-up. Thus far, repeated measurements were done in 17 patients [13 males, four females; age 58 (38–71) years at the start of treatment]. These later measurements were performed a median of 34 (19–71) months after the start of treatment. Twelve patients with a stable remission were studied, whereas five patients were studied within 4 months after onset of a relapse.

In patients with a stable remission, we observed a gradual further decrease of urinary $\beta_2$M excretion (Figure 1). In contrast, in patients with a relapse, there was a sharp increase in urinary IgG excretion from 14 to 373 mg/24 h ($P<0.05$), in urinary $\beta_2$M excretion from 0.7 to 4.9 mg/min ($n=3$; NS) and in urinary $\alpha_1$M excretion from 25.9 to 63.1 mg/min ($P<0.05$).

**Relationship between tubular reabsorption of $\beta_2$M and tubular excretion of IgG**

The data of the repeated measurements at 0, 2, 6 and 12 months after the start of treatment have allowed us to study in more detail the possibility of blockade of tubular reabsorption of $\beta_2$M by IgG. In Figure 2, we have plotted the reabsorption of $\beta_2$M against the urinary excretion of IgG [mg/100 ml glomerular filtration rate (GFR)]. It is evident that there is only a weak correlation, and it can be calculated that variations in urinary IgG excretion cannot explain the changes in urinary $\beta_2$M excretion ($r^2=0.14$). Thus, the urinary excretion of $\beta_2$M is a valuable marker of tubular dysfunction and not merely a reflection of glomerular permeability changes. Additionally, we examined the correlation between the urinary excretion of $\alpha_1$M and the urinary excretion of IgG (mg/100 ml GFR), also showing a non-significant correlation (Pearson correlation coefficient 0.276, NS). As could be expected, there was a significant correlation between the urinary excretion of $\alpha_1$M and $\beta_2$M (Pearson correlation coefficient 0.762, $P<0.01$).
Discussion

We previously have shown that the urinary excretion of the low molecular weight proteins β2M and α1M and the high molecular weight protein IgG accurately predicted renal outcome in patients with idiopathic membranous nephropathy [5–7]. In multivariate analysis, urinary β2M excretion proved to be the best independent predictive variable [7], in agreement with observations that renal function deterioration is better correlated with tubulo-interstitial injury than with glomerular damage [11]. Furthermore, we have reported that patients with idiopathic membranous nephropathy and renal insufficiency can be effectively treated with cyclophosphamide and steroids [14]. We have now evaluated the effect of this therapy on glomerular permeability and tubular proteinuria. Furthermore, we aimed to determine the predictive value of tubular and glomerular proteinuria at the end of the treatment year for long-term outcome.

The study in 11 patients, measured on four different occasions during the treatment year, confirmed the marked improvement in renal function. During the treatment year, serum albumin and serum cholesterol improved as a result of the reduction of proteinuria, with most patients entering a partial remission of proteinuria. Although ACEIs and/or ARBs, and more recently statins, have been shown to lower proteinuria [18–20], it is unlikely that the reduction of proteinuria that we observed in our patients can be completely attributed to these drugs since the use of these medications did not change during the treatment year. Thus, the improvement of renal function and proteinuria can be predominantly attributed to the immunosuppressive therapy.

In these 11 patients, immunosuppressive therapy resulted in a rapid improvement in glomerular permeability and tubulo-interstitial injury, as reflected by the lower SI and the decreased excretion of the low molecular weight proteins β2M and α1M.

Although improvement was already noted at 2 months after the start of therapy, and all but one patient were in partial remission at 12 months, some degree of tubulo-interstitial injury remained evident at 12 months. This latter finding was confirmed further by the 12 month data in the group of 25 patients. Urinary β2M was abnormal in 20 out of 21 evaluated patients and above our previously established threshold of 0.5 μg/min in 13 patients.

The observed lower values of serum IgG at 2 and 6 months after the start of therapy are most probably the result of the immunosuppressive lymphocytotoxic therapy. The urinary excretion of IgG decreased to a greater extent than the urinary excretion of albumin. This can be partly explained by the initial decrease of serum IgG but, furthermore, reflects an improvement in glomerular size selectivity as indicated by the lower SI.

In view of the high accuracy of our parameters in predicting renal function deterioration in patients with idiopathic membranous nephropathy and normal renal function, we aimed at evaluating the predictive value of these parameters for the long-term outcome after treatment. For this analysis, we could not use renal insufficiency as the end-point, since only two patients had deterioration of renal function during follow-up. This confirms the efficacy of our treatment schedule. Therefore, we have used persistence or relapse of the nephrotic syndrome as the end-point. The results of the analysis must be interpreted with some caution, since we have observed only six failures out of 25 treated patients and only 19 patients had a follow-up time of >24 months. However, the data suggest that values at the end of therapy do not predict prognosis. It is highly unlikely that even in a larger patient group and with longer follow-up, parameters will be found with high enough sensitivity and specificity. Thus, from our study, we must conclude that measurement of these various high and low molecular proteins seems to have no value when patients have been treated.

Furthermore, we have studied tubular proteinuria in patients with a longstanding stable remission. A gradual further improvement was noted after many years of follow-up, suggesting that recovery of tubulo-interstitial injury is a slow but continuous process.

We have examined in more detail the possible effects of IgG on tubular reabsorption of β2M. It is clear that the urinary IgG has only a limited effect on tubular reabsorption of β2M, if any. The same is true for the urinary excretion of α1M.

In conclusion, in patients with idiopathic membranous nephropathy, a nephrotic syndrome and renal insufficiency, treatment with cyclophosphamide and steroids not only is highly effective in inducing remissions, but also significantly improves the urinary excretion of IgG, β2M and α1M. However, levels of tubular proteinuria remain significantly abnormal at the end of the treatment year. Unfortunately, neither the values at the end of the treatment year nor the percentage reduction during the treatment year allow prediction of long-term prognosis. The increased urinary excretion of β2M in patients with a nephrotic syndrome is not the result of inhibition of tubular reabsorption by IgG and therefore seems an accurate marker of tubular damage.

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Conflict of interest statement. None declared.

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