Optimizing the treatment of idiopathic membranous nephropathy

Peggy du Buf-Vereijken

Optimizing the treatment of idiopathic membranous nephropathy

Een wetenschappelijke proeve op het gebied van de Medische Wetenschappen

Proefschrift

ter verkrijging van de graad van doctor aan de Radboud Universiteit Nijmegen
op gezag van de Rector Magnificus prof. dr. C.W.P.M. Blom,
volgens besluit van het College van Decanen
in het openbaar te verdedigen op dinsdag 29 november 2005
des namiddags om 1.30 uur precies

door

Petronella Wilhelmina Godefrida du Buf-Vereijken

geboren op 28 april 1967 te Weert

Promotor:

Prof. Dr. J.F.M. Wetzels

Manuscriptcommissie:

Prof. Dr. P.A.B.M. Smits

Prof. Dr. L.A.H. Monnens

Dr. C.A. Stegeman (UMC Groningen)

The studies presented in this thesis were performed at the Department of Nephrology of the Radboud University Nijmegen Medical Center, Nijmegen, the Netherlands, and were financially supported by the Dutch Kidney Foundation (PC 152).

Printing of this thesis is supported by a grant from the Dutch Kidney Foundation.

Thesis Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands

ISBN-10: 909020007X; ISBN-13: 9789090200071

Ontwerp omslag en binnenwerk:

Gerard du Buf & Peggy du Buf-Vereijken

Druk:

PrintPartners Ipskamp, Enschede

Aan mijn ouders

Aan Gerard, Koen en Vera

Table of contents

Chapter 1

Introduction and outline of the thesis

Introduction

The nephrotic syndrome is a clinical syndrome consisting of proteinuria of at least 3.5 g/day, low serum albumin and edema, which is often the presenting symptom. A nephrotic syndrome is always caused by a renal disease with glomerular injury. As the presenting features and laboratory investigations are not distinctive enough to permit a diagnosis, a renal biopsy is necessary to elucidate the underlying glomerular disease. Membranous nephropathy (membranous glomerulonephritis) is the most common cause of the nephrotic syndrome in adults,[1] accounting for about one third of cases. Other causes are minimal change disease, the most common cause of a nephrotic syndrome in children, focal segmental glomerulosclerosis and membranoproliferative glomerulonephritis.

Membranous nephropathy can be secondary to other diseases, like immune diseases as systemic lupus erythematosus (SLE), can be associated with infections, like hepatitis B, can be induced by drugs like gold, penicillamine and nonsteroidal anti-inflammatory drugs (NSAIDs) or can occur in relation to a malignancy. In about two-thirds of cases, however, no obvious etiologic agent or condition can be identified and the disease is called idiopathic.

Most patients with idiopathic membranous nephropathy present with a nephrotic syndrome, although proteinuria can be less severe. The natural history of patients with idiopathic membranous nephropathy is variable. In general, about half of the patients will develop a spontaneous remission of proteinuria, 10% will have persistent (mild) proteinuria and 40% will progress to renal insufficiency.[2-4]
Multiple risk factors associated with future renal function deterioration have been defined.[5] It is evident, that a steady rise in serum creatinine is the best predictor of future development of end-stage renal disease.[3;6;7] Furthermore, the persistence of severe proteinuria has been shown to be a prominent risk factor for disease progression,[8;9] requiring however a certain time of observation. We have previously presented the urinary excretion of the low molecular weight protein β_2-microglobulin[10] and the urinary excretion of IgG[11] as promising markers for disease progression.

Treatment of patients with membranous nephropathy consists of supportive treatment directed at proteinuria or the nephrotic syndrome and specific immunosuppressive therapy.

There is little discussion about the so-called conservative treatment of a patient with a nephrotic syndrome. This comprises reduction of edema with diuretics and aggressive lowering of blood pressure to values below 125/75 mm Hg with the preferential use of ACE-inhibitors or Angiotensin II type I receptor blockers, because of their additional antiproteinuric effects. In addition, lipid lowering agents (statins) are advised for the often prominent hypercholesterolemia. In case of a severe nephrotic syndrome one should consider the prescription of anticoagulant drugs to prevent thrombo-embolic complications.
In contrast to the generally accepted conservative treatment policy, there is much debate on the need for immunosuppressive agents as well as on which agent should be used in which patient at what time point. Some investigators argue against the use of any immunosuppressive drug, referring to the benign natural course in most patients.[4;12] Others have advised treatment of all patients with a membranous nephropathy with immunosuppressive agents, like chlorambucil and cyclophosphamide in combination with steroids.[13-15] Ponticelli and colleagues were the first to document improved renal survival in patients with idiopathic membranous nephropathy, treated with a regimen consisting of a six-months course of alternating cycles of steroids and chlorambucil. In this study, the probability of surviving without developing end-stage renal disease was 92% at 10 years in patients treated with immunosuppression versus 60% in untreated controls.[14] Routine use of this treatment, however, would expose 40% of patients unnecessarily to toxic immunosuppressive drugs.

Outline of the thesis

The studies presented in this thesis were aimed at defining the optimal treatment strategy for patients with idiopathic membranous nephropathy. The studies were designed to demonstrate the efficacy and safety of restricting immunosuppressive therapy to patients with idiopathic membranous nephropathy at the highest risk for disease progression. Additionally, we have validated the value of measuring the urinary excretion of β_2-microglobulin and IgG in patients with idiopathic membranous nephropathy in predicting prognosis.

We have studied the efficacy and side effects of a 12-months course of oral cyclophosphamide with steroids for the treatment of patients with idiopathic membranous

nephropathy and deteriorating renal function, thus patients at highest risk for disease progression (***Chapter 2***). Some patients do not respond or relapse after the treatment. In ***Chapter 3***, we have documented the efficacy of treating non-responders and relapsing patients with repeated courses of immunosuppression and have calculated the time gained before the onset of end-stage renal disease. Given the frequent and severe side effects associated with the treatment with cyclophosphamide, we have started a pilot study, in 2002, to assess the efficacy of mycophenolate mofetil for the treatment of patients with idiopathic membranous nephropathy and deteriorating renal function. We report the short-term efficacy and side effects, based on the experience in thirteen patients (***Chapter 4***).

Since 1988, we have advised to restrict immunosuppressive therapy to patients with a debilitating persistent nephrotic syndrome or renal function deterioration. To support the safety of our restrictive treatment policy we have performed a prospective cohort study which included all patients biopsied in the period 1988-2002 with normal renal function at the time of renal biopsy. The results of this study are presented in ***Chapter 5***.

To allow starting immunosuppressive therapy before the onset of renal failure, it is essential to be able to predict prognosis early in the course of the disease. In ***Chapter 6*** we have evaluated the accuracy of the urinary excretion of β_2-microglobulin and IgG as parameters predicting disease progression in patients with normal renal function. Unfortunately, β_2-microglobulin can only be measured in urine if urinary pH exceeds 6.0. Since this is not the case in about 7% of patients, despite the use of sodium bicarbonate, we investigated the value of acetazolamide to alkalinize urine in ***Chapter 7***.
In view of the high predictive value of the urinary excretion of β_2-microglobulin and IgG in patients with idiopathic membranous nephropathy and normal renal function, we questioned the value of measuring these parameters during and at the end of therapy in patients who received cyclophosphamide and steroids because of renal function deterioration (***Chapter 8***).

Based upon our experience and a review of the literature we propose a rational treatment strategy for patients with idiopathic membranous nephropathy in ***Chapter 9***.

References

1. Haas M, Meehan SM, Karrison TG, Spargo BH. Changing etiologies of unexplained adult nephrotic syndrome: a comparison of renal biopsy findings from 1976-1979 and 1995-1997. *Am J Kidney Dis* 1997; 30: 621-631
2. MacTier R, Boulton Jones JM, Payton CD, McLay A. The natural history of membranous nephropathy in the West of Scotland. *Q J Med* 1986; 60: 793-802
3. Donadio JV, Jr, Torres VE, Velosa JA, *et al*. Idiopathic membranous nephropathy: the natural history of untreated patients. *Kidney Int* 1988; 33: 708-715
4. Schieppati A, Mosconi L, Perna A, *et al*. Prognosis of untreated patients with idiopathic membranous nephropathy. *N Engl J Med* 1993; 329: 85-89
5. Reichert LJ, Koene RA, Wetzels JF. Prognostic factors in idiopathic membranous nephropathy. *Am J Kidney Dis* 1998; 31: 1-11
6. Honkanen E, Tornroth T, Gronhagen-Riska C, Sankila R. Long-term survival in idiopathic membranous glomerulonephritis: can the course be clinically predicted? *Clin Nephrol* 1994; 41, 127-134
7. Torres A, Dominguez-Gil B, Carreno A, *et al*. Conservative versus immunosuppressive treatment of patients with idiopathic membranous nephropathy. *Kidney Int* 2002; 61: 219-227
8. Pei Y, Cattran D, Greenwood C. Predicting chronic renal insufficiency in idiopathic membranous glomerulonephritis. *Kidney Int* 1992; 42: 960-966
9. Cattran DC, Pei Y, Greenwood CM, *et al*. Validation of a predictive model of idiopathic membranous nephropathy: its clinical and research implications. *Kidney Int* 1997; 51: 901-907
10. Reichert LJ, Koene RA, Wetzels JF. Urinary excretion of β_2-microglobulin predicts renal outcome in patients with idiopathic membranous nephropathy. *J Am Soc Nephrol* 1995; 6: 1666-1669
11. Reichert LJ, Koene RA, Wetzels JF. Urinary IgG excretion as a prognostic factor in idiopathic membranous nephropathy. *Clin Nephrol* 1997; 48: 79-84
12. Schieppati A, Ruggenenti P, Perna A, Remuzzi G. Nonimmunosuppressive therapy of membranous nephropathy. *Semin Nephrol* 2003; 23: 333-339
13. Ponticelli C, Zucchelli P, Passerini P, *et al*. A randomized trial of methylprednisolone and chlorambucil in idiopathic membranous nephropathy. *N Engl J Med* 1989; 320: 8-13
14. Ponticelli C, Zucchelli P, Passerini P, *et al*. A 10-year follow-up of a randomized study with methylprednisolone and chlorambucil in membranous nephropathy. *Kidney Int* 1995; 48: 1600-1604
15. Ponticelli C, Altieri P, Scolari F, *et al*. A randomized study comparing methylprednisolone plus chlorambucil versus methylprednisolone plus cyclophosphamide in idiopathic membranous nephropathy. *J Am Soc Nephrol* 1998; 9: 444-450

Chapter 2

Cytotoxic therapy for membranous nephropathy and renal insufficiency: improved renal survival but high relapse rate

Peggy W.G. du Buf-Vereijken, Amanda J.W. Branten and Jack F.M. Wetzels
for the Membranous Nephropathy Study Group

Department of Nephrology, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands

Nephrol Dial Transplant 2004; 19(5): 1142-8

Abstract

Background. Patients with idiopathic membranous nephropathy (iMN) and renal insufficiency have a high risk for progression to end-stage renal disease (ESRD). In the short term, treatment with oral cyclophosphamide and steroids attenuates the deterioration of renal function in these patients; however, the long-term efficacy is unknown.

Methods. We have studied prospectively 65 patients with iMN and renal insufficiency (serum creatinine > 135 μmol/l) who were treated with oral cyclophosphamide (1.5-2.0 mg/kg/day for 12 months) and steroids (methylprednisolone pulses 3x1 g, i.v. at months 0, 2 and 4 and oral prednisone 0.5 mg/kg/48h for 6 months).

Results. Follow-up was 51 (5-132) months. Renal function temporarily improved or stabilized in all patients. A partial remission (PR) occurred in 56 patients followed by a complete remission (CR) in 17. During follow-up, 11 patients had relapsed (28% relapse rate after 5 years), of whom nine were re-treated because of renal function deterioration. At the end of follow-up, 16 patients were in CR, 31 in PR, eight had a persistent nephrotic syndrome, one had mild proteinuria, four had progressed to ESRD and five had died. Overall renal survival was 86% after 5 years and 74% after 7 years, compared with 32% after 5 and 7 years in a historical control group. Treatment-related complications occurred in two-thirds of patients, mainly consisting of bone marrow depression and infections. One patient has developed bladder cancer, another patient prostate cancer.

Conclusions. Renal survival is good if patients with iMN and renal insufficiency are treated with oral cyclophosphamide. However, side effects occur frequently and relapse rate is high during longer follow-up.

Introduction

Idiopathic membranous nephropathy remains the most common cause of the nephrotic syndrome in adults.[1] Studies on the natural history of the disease show that up to 40% of untreated patients will progress to end-stage renal disease (ESRD).[2-5]

The immunosuppressive treatment of idiopathic membranous nephropathy is still a matter of debate. Some authors advocate immunosuppressive treatment for all patients with idiopathic membranous nephropathy and nephrotic syndrome, based on a randomized controlled trial conducted in Italy, clearly demonstrating that treatment with a combination of chlorambucil and prednisone improves renal survival in patients with idiopathic membranous nephropathy.[4] However, other authors argue rather strongly against the need for immunosuppressive treatment in view of the observed benign course in > 50% of patients.[3] Therefore, we and others are in favour of restricting immunosuppressive therapy to patients at highest risk of developing ESRD. (reviewed in[6])[5;7-11]

In patients with idiopathic membranous nephropathy, various risk factors for the development of renal failure have been identified (reviewed by Reichert *et al.*[12]). However, the sensitivity and specificity of most factors is too low to justify their use to guide decisions on the start of immunosuppressive therapy.[12] It is evident, however, that an established deterioration of renal function is a powerful predictor of ESRD.[2;8;10;12] Therefore, most would agree that a trial of immunosuppressive therapy is warranted in such patients with idiopathic membranous nephropathy and renal function deterioration. We previously have shown that immunosuppressive treatment can attenuate the deterioration of renal function in these patients.[6;7] In this group of patients, treatment with oral cyclophosphamide and steroids seemed more effective and less toxic than the combination of chlorambucil and steroids (overview of the literature data in[6]). Admittedly, there are no controlled trials that document the efficacy of immunosuppressive therapy in patients with idiopathic membranous nephropathy and established renal insufficiency. Furthermore, most data are derived from small studies with short follow-up (overview of the literature in[6]).[5;7;10] A recent study demonstrated that the renal outcome was better in patients with idiopathic membranous nephropathy and renal failure who were treated with the combination of chlorambucil and prednisone when compared with historical controls.[10]

Since June 1991, we have prospectively studied patients with idiopathic membranous nephropathy and renal insufficiency. The data of these patients, who have been treated with

oral cyclophosphamide and steroids, form the basis of this report. Our study comprises the largest patient cohort described so far.

Subjects and Methods

We included only adult patients (age > 18 years) with a biopsy-proven membranous nephropathy in whom a secondary cause of membranous nephropathy was excluded on clinical and/or laboratory grounds. Patients were recruited in our University Hospital or in one of the 19 referring hospitals. Eligible patients had to have evidence of renal insufficiency (defined as a serum creatinine > 135 μmol/l, a calculated endogenous creatinine clearance < 70 ml/min or a rise in serum creatinine of > 50%) and a proteinuria of at least 2.0 g/10 mmol creatinine. Exclusion criteria were systemic diseases, malignancies, active infection, pregnancy or inadequate contraconception, unstable angina pectoris, diabetes mellitus type I or long-lasting diabetes mellitus type II, clinical evidence of renal vein thrombosis, liver test abnormalities (> 2x upper limit of normal), active peptic ulcer disease or gastro-intestinal diseases that could impair the resorption of oral medication. Patients who used immunosuppressive therapy in the previous 6 months were not eligible, except in the case of evident failure of treatment.

Details of the immunosuppressive treatment have been described.[7] In brief, treatment consisted of 1.5-2.0 mg/kg/day of oral cyclophosphamide, for 1 year, 1 g of methylprednisolone i.v. for three consecutive days at the beginning of the first, third and fifth month, and 0.5 mg/kg of oral prednisone every other day for 6 months with subsequent tapering. For the prevention of gastric complaints, famotidine was added, and to prevent *Pneumocystis carinii* pneumonia, most patients received 480 mg of trimethoprim-sulfamethoxazole daily in the first 4-6 months. All patients were advised to follow a moderately salt-restricted diet. Conservative treatment was not standardized; however, physicians were instructed to lower blood pressure aggressively. More recently, it has become practice to use angiotensin-converting enzyme (ACE) inhibitors and/or angiotensin receptor blockers for all patients with proteinuria, and to add cholesterol-lowering therapy. Anticoagulant drugs were not prescribed routinely.

For survival analysis, the time of follow-up started at the beginning of treatment with cyclophosphamide and steroids. Follow-up continued until September 2002, or ended at the time of death or the onset of ESRD.

Patients were seen regularly during follow-up, every 4-8 weeks during treatment and every 3-4 months thereafter, but less frequently in cases where complete remission (CR) occurred. Blood pressure, complications of the nephrotic syndrome, side effects of the therapy and laboratory data were registered. To correct for inappropriate 24 h urine collections, the amount of proteinuria was expressed as a protein-creatinine index (g/10 mmol creatinine). A CR of proteinuria, partial remission (PR), 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. All patients who entered a CR were also registered as having a partial remission. Relapses were defined as nephrotic range proteinuria after a PR or CR 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.

A second course of immunosuppressive therapy was offered to patients who relapsed to nephrotic range proteinuria together with a rise in serum creatinine of > 50% over the lowest value attained during or after the first course of cyclophosphamide treatment.

The historical controls (*n*=24) consisted of patients with an idiopathic membranous nephropathy and renal insufficiency (serum creatinine > 135 μmol/l) referred to our University Hospital for therapeutic advise or inclusion in therapeutic trials, and thus came to our attention in the same way as the patients included in this cohort study. Several of these historical control patients were included in former trials and treated with prednisone monotherapy (*n*=7), i.v. cyclophosphamide (*n*=1) or both (*n*=3). Because these treatment modalities have proved ineffective,[9;13] these patients can be considered historical controls. Most of the historical control patients were not treated with immunosuppressive therapy at all (*n*=13), mainly because we were not used to do so before June 1991.

Calculations and statistics

For descriptive statistics, results are given as means ± SD, or medians with range when appropriate. Mean arterial blood pressure (MAP) was calculated using de formula MAP = diastolic blood pressure + 1/3 x (systolic blood pressure - diastolic blood pressure). For calculations of renal survival, the time of renal death was defined as the start of renal

replacement therapy or the time of death. The cumulative probabilities of a clinical event (death, ESRD, PR, CR or relapse of nephrotic syndrome) were estimated according to Kaplan and Meier. The log rank test was used to compare survival curves. To demonstrate further an effect of the immunosuppressive therapy on the rate of deterioration of renal function, we have calculated the slope of 1000/serum creatinine *vs* time before and after the start of the treatment for the treated patients and overall for the historical controls. The Mann-Whitney test was used for comparison between groups, and Wilcoxon signed rank test for comparisons within the group of treated patients. A *P*-value of < 0.01 was considered significant. All statistical procedures were done using SPSS software (SPSS version 10.0, Chicago, IL, USA).

Results

Baseline characteristics of the treated patients are given in Table 1.

Table 1. Baseline characteristics

Number of patients	65
Male : female	55 : 10
Age at clinical onset of disease (years)	50 (15-77)
Age at time of biopsy (years)	51 (15-77)
Interval between biopsy and start of cyclophosphamide (months)	13 (0-280)
Serum creatinine (μmol/l)	171 (106-512)
Serum albumin (g/l)	23 (9-43)
Proteinuria (g/10 mmol creatinine)	10.0 (2.0-23.0)
Creatinine clearance (ml/min/1.73 m^2)	42 (13-109)
Mean arterial pressure (mm Hg)	102 (80-133)
Follow-up (months)	51 (5-132)

Values are medians with range.

Most patients (n=59) started immunosuppressive therapy because of a serum creatinine > 135 μmol/l, and six patients started immunosuppressive therapy because of a 50% increase in

serum creatinine. Calculated endogenous creatinine clearance (Cockcroft formula) in the latter group was 86 (51-109) ml/min/1.73 m^2, proteinuria 10.2 (4.5-16.0) g/10 mmol creatinine and serum albumin 23 (17-30) g/l. Inclusion of these patients did not influence the results.

Nineteen patients (29%) had received previous immunosuppressive therapy, mainly consisting of short-term high-dose prednisone (n=7), prednisone followed by a combination of prednisone and chlorambucil (n=3) or only a combination of prednisone and chlorambucil (n=6). Four patients had received immunosuppressive therapy < 6 months before the start of cyclophosphamide treatment, but this previous treatment had failed as evidenced by progressive renal insufficiency. Follow-up after the start of therapy averaged 51 months (SD 30 months, range 5-132 months). Twenty-one patients have been followed for > 5 years.

Median values of MAP, serum creatinine, serum albumin, serum cholesterol, proteinuria and creatinine clearance, before, at regular intervals after the start of therapy and at the end of follow-up, are given in Table 2.

Table 2. Laboratory parameters and blood pressure during treatment and follow-up

	-6 months (n =50)	Start therapy (n =65)	3 months (n =65)	12 months (n =60)	24 months (n =51)	End follow-up (n =65)
Screatinine (µmol/l)	129** (68-376)	171 (106-512)	130** (73-642)	120** (74-337)	123** (85-370)	128** (69-1000)
Salbumin (g/l)	22 (13-42)	23 (9-43)	29** (11-42)	38** (25-47)	40** (23-50)	39** (22-46)
Scholesterol (mmol/l)	7.7 (5.0-28.8)	7.6 (4.6-23.2)	6.9** (4.4-14.0)	5.5** (3.8-8.5)	5.4** (3.1-7.1)	5.2** (3.1-10.3)
Proteinuria (g/10 mmol creat)	8.9* (3.4-17.9)	10.0 (2.0-23.0)	4.8** (0-34.2)	1.3** (0-13.0)	0.8** (0-8.5)	0.8** (0-11.1)
Creatinine clearance (ml/min/1.73 m^2)	61** (22-170)	42 (13-109)	59** (15-143)	62** (17-136)	60** (16-138)	61** (7-155)
MAP (mm Hg)	92 (69-118)	102 (80-133)	94** (65-125)	95** (75-124)	97* (77-122)	93* (71-118)

Values are medians with range.
S, serum; MAP, mean arterial pressure.
* $P < 0.01$, ** $P < 0.001$, compared with values at the start of treatment.

At the start of cyclophosphamide treatment blood pressure was reasonably well controlled, and 78% of patients was treated with an ACE-inhibitor and/or angiotensin receptor blocker.

Blood pressure further improved during follow-up, although antihypertensive treatment was not intensified.

Serum creatinine improved by > 10% in 91% of patients during the first year. In only three patients (4.6%) was serum creatinine increased by > 10% during the treatment year. The lowest serum creatinine value attained after the start of treatment was 107 (70-489) µmol/l and this nadir was reached after a median of 6 months. Immunosuppressive therapy attenuated the deterioration of renal function as indicated by the change in the median slope of 1000/serum creatinine from –3.0 l/µmol/yr before to 0.12 l/µmol/yr after the start of therapy ($P < 0.001$).

Proteinuria also decreased in the majority of patients. This decrease in proteinuria was gradual, and continued even after the end of the immunosuppressive treatment (Table 2). As can be expected, serum albumin improved and cholesterol declined after the start of treatment (Table 2).

Overall, 56 patients have developed a PR of proteinuria after an average of 10.6 months. In 17 patients, proteinuria further decreased to values ≤ 0.2 g, and this CR was reached 12 (0-38) months after the onset of the PR. The cumulative incidence of PR is 92% after 5 years and of CR is 36% after 5 years (Figure 1).

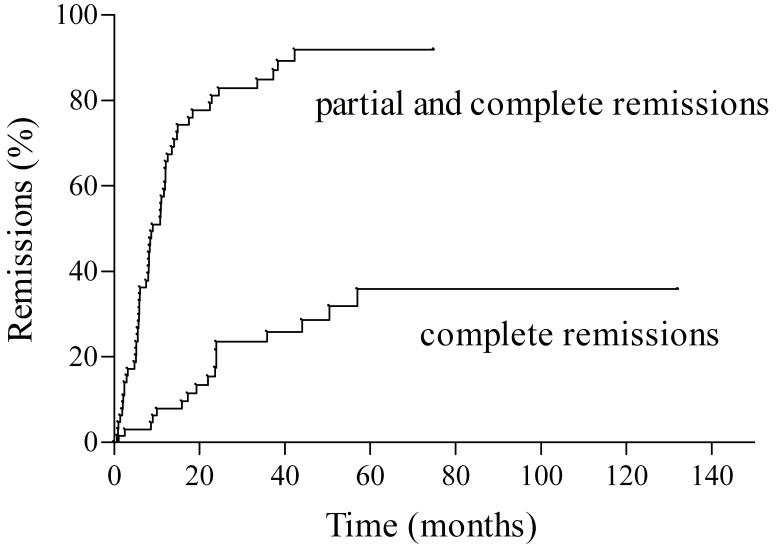


Figure 1. Cumulative incidence of partial and complete remissions of proteinuria.

Not all patients have remained in stable remission. Of the 17 patients who developed a CR, one relapsed to nephrotic range proteinuria. Of the 39 patients who have developed only a PR of proteinuria, 10 relapsed to nephrotic range proteinuria. Relapsing patients had been in

remission for 27 (7-66) months. The cumulative incidence of relapses is 28% at 5 years after onset of the remission (Figure 2).

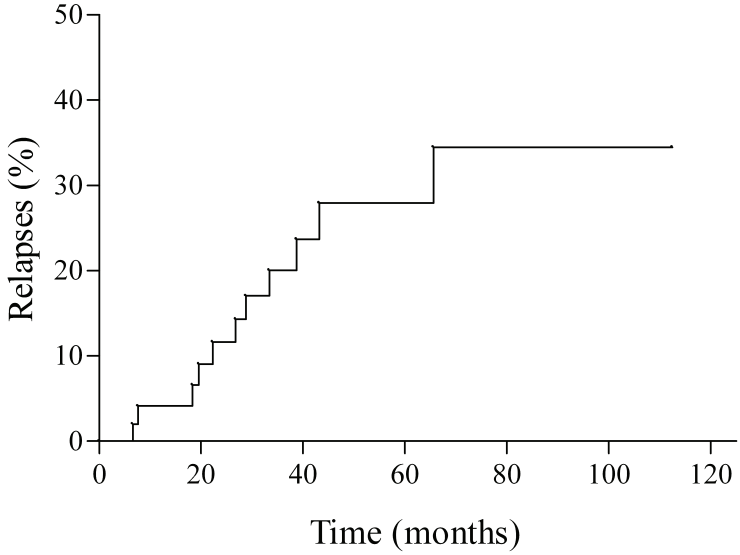


Figure 2. Cumulative incidence of relapses after remission.

In most relapsing patients, serum creatinine eventually has increased by > 50%. The occurrence of a relapse could not be predicted by the serum creatinine, the amount of proteinuria or MAP at the start of therapy.

The overall course in our patient group and the status at the end of follow-up are depicted in Figure 3. Thus far, nine patients have been retreated because of a relapse and again deteriorating renal function. At the end of the follow-up, 16 patients were in CR, 31 patients in PR, eight patients have a persistent nephrotic syndrome and one patient has mild persistent proteinuria. Four patients have reached ESRD; in three of these, serum creatinine was ≥ 400 µmol/l at the start of treatment. Five patients have died at a median of 2.7 years after the start of therapy.

Of the group of patients that started immunosuppressive therapy because of a 50% rise in serum creatinine (n=6) two patients were in CR (33%), three in PR (50%) and one had a persistent nephrotic syndrome (17%).

Outcome was similar in patients who had received immunosuppressive therapy prior to starting cyclophosphamide (n=19) and patients who had not been treated before. Also, when analysing the data separately for patients with a serum creatinine below or above the median value of 171 µmol/l at start of therapy, no differences in outcome were noted.

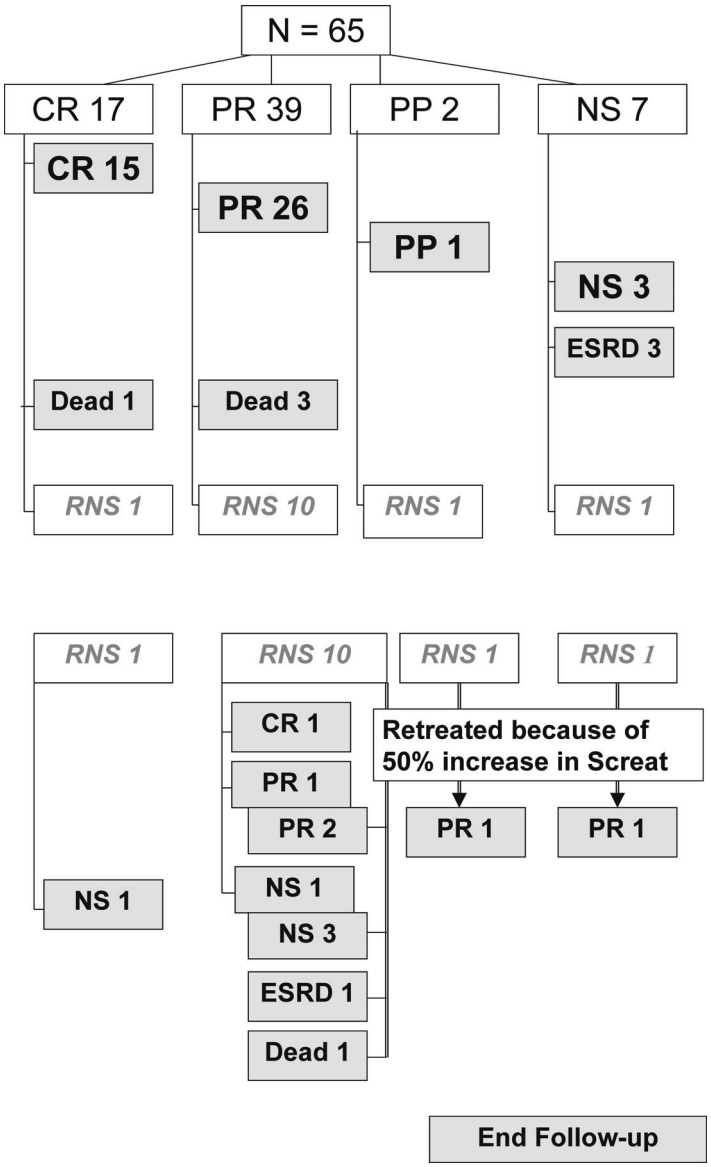


Figure 3. Flow-chart of treatment results**.**

CR, Complete Remission = proteinuria ≤ 0.2 g/10 mmol creatinine;
PR, Partial Remission = proteinuria 0.21-2.0 g/10 mmol creatinine;
PP, Persisting Proteinuria = proteinuria 2.1-3.4 g/10 mmol creatinine;
(R)NS, (Recurrent) Nephrotic Syndrome = proteinuria ≥ 3.5 g/10 mmol creatinine;
ESRD, end-stage renal disease.
Status at the end of follow-up is given in the gray rectangles.

A PR or CR of proteinuria developed in 43 out of 51 patients using an ACE inhibitor or angiotensin receptor antagonist as compared with 13 out of 14 patients who did not use these agents (*P* = NS). The use of an ACE inhibitor or angiotensin receptor antagonist also did not influence renal survival rate or death (7/51 *vs* 2/14; *P* = NS). We also did not observe an effect of blood pressure values on remission rate or renal outcome.

In our study, renal survival without censoring for death is 86% at 5 years and 74% at 7 years after the start of treatment. We have compared renal survival in our treated patients with renal survival in a group of 24 historical controls. In these historical controls, renal survival was 32% at 5 years, a highly significant difference ($P < 0.001$; Figure 4). Results are similar if only untreated control patients are included in the analysis.

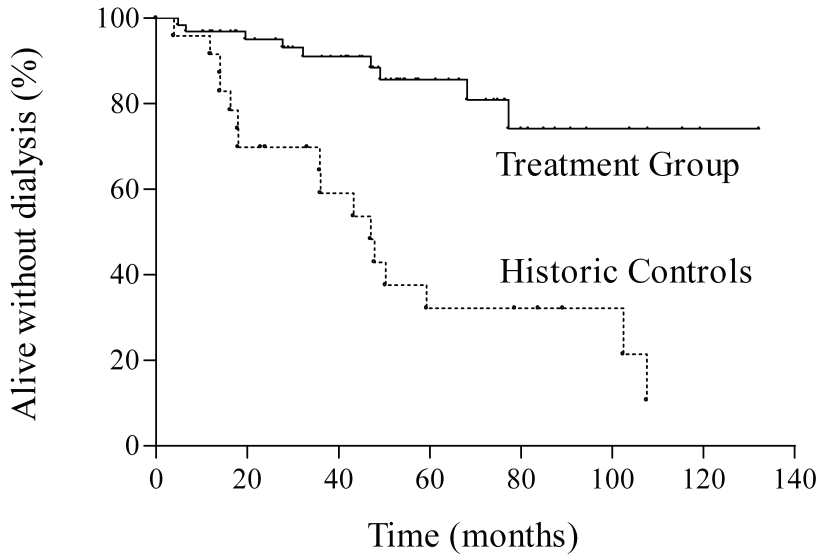


Figure 4. Renal Survival.

Renal survival for treated patients (n=65) was 86% after 5 years and 74% after 7 years; for historic controls (n=24), it was 32% after 5 years, the difference being significant ($P < 0.0001$).

The beneficial effect of cyclophosphamide therapy in attenuating deterioration of renal function is also obvious if we compare the slopes of 1000/serum creatinine: 0.12 l/μmol/year with cyclophosphamide compared with –1.21 l/μmol/year in the historical controls ($P < 0.001$).

Five patients have died, at a median age of 63 (43-79) years. Two patients died suddenly, at home of unknown causes while in CR or PR. One patient died from cardiovascular disease while being re-treated. One patient died due to sepsis, in CR 7 months after the completion of immunosuppressive treatment. The fifth patient died of a disseminated bladder carcinoma, which he developed 21 months after the start of therapy. This patient had received a cumulative dose of 20 g of cyclophosphamide. Patient survival is 91% after 5 years and 84% after 7 years.

Side effects were frequent. Of 65 patients, 43 experienced one or more side effects during the treatment year. Bone marrow depression and infectious complications were most frequent (Table 3). In most patients, the dose of cyclophosphamide was temporarily reduced (Table 3). Only four patients (6%) had to stop cyclophosphamide within 6 months after the start of treatment; in two patients, azathioprine was used as replacement.

Table 3. Treatment-related complications

Complication / side effect	Number of patients (%)	Temporary dose reduction (%)	Treatment ended < 6 months (%)
Bone marrow depression			
Leucocytopenia	27 (42%)	19 (29%)	1 (1.5%)
Anaemia	10 (15%)	6 (9%)	1 (1.5%)
Thrombocytopenia	3 (4.6%)	2 (3%)	0 (0%)
Infections			
All infections	17 (26%)	6 (9%)	1 (1.5%)
Respiratory infections	11 (17%)	3 (4.6)	0 (0%)
Malaise	8 (12%)	5 (7.7%)	2 (3%)
Liver test abnormalities	2 (3%)	2 (3%)	1 (1.5%)
Steroid-induced diabetes mellitus	1 (1.5%)	0 (0%)	0 (0%)
Total number of patients	43 (66%)	30 (46%)	4 (6%)

The numbers do not add up, as one patient can have more than one complication.
Definitions: leucocytopenia: leucocyte count < 3.0 x 10^9/l; anaemia: haemoglobin < 6.0 mmol/l; thrombocytopenia: thrombocyte count < 100 x 10^9/l.

Thromboembolic complications occurred in three patients after the start of treatment. Two patients have developed a malignancy after treatment: one patient developed a bladder carcinoma (see above) and one patient a prostate carcinoma.

Discussion

Our study clearly demonstrates that treatment with oral cyclophosphamide and prednisone improves renal survival in patients with idiopathic membranous nephropathy and renal insufficiency. Admittedly, we have not performed a randomized, controlled trial.

However, our study represents the largest cohort of treated patients studied prospectively over a long time period. If we compare renal survival in our treated patients with that in a group of historical controls from our centre, there is a clear survival difference (5 year renal survival 86% *vs* 32%). We have also compared our results with data reported by other investigators. In untreated patients with idiopathic membranous nephropathy and renal insufficiency, reported renal survival rates range from 20 to 30% after 7 years.[8;10] Moreover, our renal survival rate is even higher than those reported for untreated patients with idiopathic membranous nephropathy and normal renal function.[2;4] Our study thus supports the conclusions from recent smaller studies that immunosuppressive therapy improves outcome in patients with idiopathic membranous nephropathy and renal failure.[8;10] Apparently, these conclusions do not hold for all immunosuppressive regimens containing cytotoxic drugs. Falk *et al*. have reported that i.v. cyclophosphamide did not offer additional benefits.[9] In a randomized study, we also demonstrated that i.v. cyclophosphamide was not effective.[13] Azathioprine with oral prednisone, without methylprednisolone infusions, has also been used without success, as reported in a retrospective study.[14] In contrast, chlorambucil has been used with apparent success.[5;6;10] However, in our experience, chlorambucil may be less effective than cyclophosphamide and causes more side effects.[6]

Based on the findings of the randomized trials conducted by Ponticelli *et al.*[4] and our present and previous observations, we feel that it is no longer justified to withhold treatment from patients with idiopathic membranous nephropathy and renal failure. Therefore, it might be impossible to perform a placebo-controlled trial. Although the results of our study seem favourable, we cannot definitively answer the question of whether the start of immunosuppressive therapy can be delayed until renal insufficiency develops. In the study of Ponticelli *et al.,* 10 year renal survival was 92%, a value which is better than our 74% renal survival after 7 years. However, patients were not comparable at all since we included only high-risk patients whereas in the Italian randomized study patients were included with a lower predicted risk, as indicated by the short duration of disease and the almost normal renal function at the start of therapy. Furthermore, we have calculated renal survival from the time of starting immunosuppressive therapy, which evidently causes an underestimation of survival rate. If we calculate renal survival from the time of renal biopsy, estimated 5, 7 and 10 year renal survival rates are 93%, 90% and 81% respectively.

Thus far, five patients have died. No patient died of renal failure. In one patient, who died from bladder carcinoma, death may have been related to treatment. The other patients died long after the end of treatment, most frequently from cardiovascular causes. Patient survival of 91% at 5 years and 84% at 7 years is comparable with or better than reported data.[2;4;5;10]

Our study also illustrates some major drawbacks of our immunosuppressive protocol, i.e. a relatively high rate of relapses, frequent side effects, and lack of effectiveness in some patients.

Overall, a quarter of the patients entered a CR during follow-up. It is of note that in most patients, the time of onset of CR was > 12 months after the start of treatment, i.e. well after stopping immunosuppressive therapy. Prognosis is excellent in patients who have developed a CR, since thus far only one of these patients (6%) has relapsed. In contrast, relapses have occurred frequently in patients who responded to treatment with a PR (10 out of 39 patients, 26%) or a > 50% reduction in proteinuria, and may even increase with longer follow-up. Cumulative incidence of relapses after the occurrence of a PR or CR is 28% after 5 years. We find this relapse rate rather high; however, similar figures (30% after 2 years) have been reported by Ponticelli *et al.* for patients with membranous nephropathy and preserved renal function treated with either cyclophosphamide or chlorambucil.[15] In most of our patients who relapsed, renal function has deteriorated, necessitating a new course of immunosuppression.

Many patients experienced complications from the immunosuppressive therapy, especially bone marrow depression and infectious complications. This high incidence of side effects may be partly related to the decreased renal function of our patients.[5-7] In only 6% of patients did the severity of side effects necessitate a premature termination of therapy (< 6 months). One feared side effect of cyclophosphamide therapy is the development of neoplasias, in particular bladder cancer.[16;17] It has been shown that the risk of cyclophosphamide-related bladder cancer increases with the duration (especially > 2.7 years) and the cumulative dosage (mainly > 100g) of cyclophosphamide treatment. Bladder carcinoma can become manifest even after a latency period of more than a decade.[16;18]

The side effects of alkylating agents increases the urgency of the search for less toxic therapies. Recently, reports have become available regarding the treatment of patients with therapy-resistant or relapsing idiopathic membranous nephropathy with mycophenolate mofetil. Short-term results show substantial reductions of proteinuria, although remissions were scarce, mostly accompanied by preservation of renal function. Furthermore, mycophenolate mofetil was well tolerated.[19;20] Further results are awaited.

We feel that our data are sufficient to support the proposal of a formal comparison between early and late start of immunosuppressive therapy. Admittedly, an earlier start of immunosuppressive therapy poses a risk to some patients who would have developed a spontaneous remission of proteinuria. However, an earlier start of treatment might result in even better renal survival rates, may be associated with fewer and less severe side effects, and might result in a lower rate of relapses. Patients to be included in such studies could be selected based on defined risk factors for progressive disease such as the duration and magnitude of proteinuria or the urinary excretion of IgG, β_2-microglobulin or α_1-microglobulin.[12]

In conclusion, treatment of patients with idiopathic membranous nephropathy and renal insufficiency with oral cyclophosphamide and steroids results in a high remission rate and good renal survival, suggesting that immunosuppressive treatment is still effective when started at a time point when renal insufficiency has developed. We cannot answer the question of whether an earlier start of treatment would have been more beneficial. A formal study is warranted. Our treatment schedule with cyclophosphamide and steroids is hampered by the frequent occurrence of side effects. Unfortunately, relapses are also frequent with longer follow-up. Therefore, we need to continue studies in search of safer and more effective therapeutic agents.

Acknowledgements

A. B. is supported by a grant from the Dutch Science Foundation (NWO-MW 920-03-038).

The following colleagues have actively participated in this study of the Membranous Nephropathy Study Group:

Dr. A.J. Apperloo, St. Elisabeth Hospital, Tilburg; Professor dr. J.H.M. Berden and Dr. H.W. van Hamersvelt, Radboud University Nijmegen Medical Center, Nijmegen; Drs. J.J. Beutler, A. Hollander, J.L.J. Jansen and M.I. Koolen, Jeroen Bosch Hospital, 's Hertogenbosch; Dr. P.A. Bleeker, Hospital Rivierenland, Tiel; Drs. F.H. Bosch and K.J. Parlevliet, Rijnstate Hospital, Arnhem; Dr. M.A.G.J. ten Dam, Canisius Wilhelmina Hospital, Nijmegen; Dr. P. Douwes-Draaijer, Catharina Hospital, Eindhoven; Drs. G.W. Feith and M. den Hartog, Hospital Gelders Vallei, Ede; Dr. L.A.M. Frenken, Atrium Medical Center, Heerlen; Drs. W. Grave and J.J.J.M. Wirtz, Laurentius Hospital, Roermond; Dr. J.G.M. Jordans, Medical Spectrum Twente, Enschede; Dr. A.G. Lieverse, Máxima Medical Center, Eindhoven; Drs. A.J. Luik and C.M.C. Verstappen, Hospitals Noord-Limburg, Venlo; Drs. T.C. Noordzij and H.P.C. van Roermund, Franciscus Hospital, Roosendaal; Dr. R.E.H. Smeets, St. Anna Hospital, Geldrop; Dr. P.F.M.J. Spooren, TweeSteden Hospital, Tilburg; Dr. R. L. Stuyver, Hospital Velp, Velp; Dr. G.P. Verburg, Amphia Hospital, Breda; Dr. C.G. Vermey, Deventer Hospital, Deventer; Dr. A.W.L. van den Wall Bake, Máxima Medical Centrum, Veldhoven.

References

1. Haas M, Meehan SM, Karrison TG, Spargo BH. Changing etiologies of unexplained adult nephrotic syndrome: a comparison of renal biopsy findings from 1976-1979 and 1995-1997. *Am J Kidney Dis* 1997; 30: 621-631
2. Donadio JV, Jr, Torres VE, Velosa JA, *et al*. Idiopathic membranous nephropathy: the natural history of untreated patients. *Kidney Int* 1988; 33: 708-715
3. Schieppati A, Mosconi L, Perna A, *et al*. Prognosis of untreated patients with idiopathic membranous nephropathy. *N Engl J Med* 1993; 329: 85-89
4. Ponticelli C, Zucchelli P, Passerini P, *et al*. A 10-year follow-up of a randomized study with methylprednisolone and chlorambucil in membranous nephropathy. *Kidney Int* 1995; 48: 1600-1604
5. Stirling CM, Simpson K, Boulton-Jones JM. Immunosuppression and outcome in idiopathic membranous nephropathy. *Q J Med* 1998; 91: 159-164
6. Branten AJ, Reichert LJ, Koene RA, Wetzels JF. Oral cyclophosphamide versus chlorambucil in the treatment of patients with membranous nephropathy and renal insufficiency. *Q J Med* 1998; 91: 359-366
7. Branten AJ, Wetzels JF. Short- and long-term efficacy of oral cyclophosphamide and steroids in patients with membranous nephropathy and renal insufficiency. *Clin Nephrol* 2001; 56: 1-9
8. Jindal K, West M, Bear R, Goldstein M. Long-term benefits of therapy with cyclophosphamide and prednisone in patients with membranous glomerulonephritis and impaired renal function. *Am J Kidney Dis* 1992; 19: 61-67
9. Falk RJ, Hogan SL, Muller KE, Jennette JC. Treatment of progressive membranous glomerulopathy. A randomized trial comparing cyclophosphamide and corticosteroids with corticosteroids alone. The Glomerular Disease Collaborative Network. *Ann Intern Med* 1992; 116: 438-445
10. Torres A, Dominguez-Gil B, Carreno A, *et al*. Conservative versus immunosuppressive treatment of patients with idiopathic membranous nephropathy. *Kidney Int* 2002; 61: 219-227
11. Cattran DC. Idiopathic membranous glomerulonephritis. *Kidney Int* 2001; 59: 1983-1994
12. Reichert LJ, Koene RA, Wetzels JF. Prognostic factors in idiopathic membranous nephropathy. *Am J Kidney Dis* 1998; 31: 1-11
13. Reichert LJ, Huysmans FT, Assmann K, Koene RA, Wetzels JF. Preserving renal function in patients with membranous nephropathy: daily oral chlorambucil compared with intermittent monthly pulses of cyclophosphamide. *Ann Intern Med* 1994; 121: 328-333
14. Ahuja M, Goumenos D, Shortland JR, Gerakis A, Brown CB. Does immunosuppression with prednisolone and azathioprine alter the progression of idiopathic membranous nephropathy? *Am J Kidney Dis* 1999; 34: 521-529
15. Ponticelli C, Altieri P, Scolari F, *et al*. A randomized study comparing methylprednisolone plus chlorambucil versus methylprednisolone plus cyclophosphamide in idiopathic membranous nephropathy. *J Am Soc Nephrol* 1998; 9: 444-450
16. Talar-Williams C, Hijazi YM, Walther MM, *et al*. Cyclophosphamide-induced cystitis and bladder cancer in patients with Wegener granulomatosis. *Ann Intern Med* 1996; 124: 477-484

17. Knight A, Askling J, Ekbom A. Cancer incidence in a population-based cohort of patients with Wegener's granulomatosis. *Int J Cancer* 2002; 100: 82-85

18. Travis LB, Curtis RE, Glimelius B, *et al*. Bladder and kidney cancer following cyclophosphamide therapy for non-Hodgkin's lymphoma. *J Natl Cancer Inst* 1995; 87: 524-530

19. Miller G, Zimmerman R, III, Radhakrishnan J, Appel G. Use of mycophenolate mofetil in resistant membranous nephropathy. *Am J Kidney Dis* 2000; 36: 250-256

20. Choi MJ, Eustace JA, Gimenez LF, *et al*. Mycophenolate mofetil treatment for primary glomerular diseases. *Kidney Int* 2002; 61: 1098-1114

Chapter 3

Efficacy of a second course of immunosuppressive therapy in patients with membranous nephropathy and persistent or relapsing disease activity

Peggy W.G. du Buf-Vereijken[1,2], Jack F.M. Wetzels[1]

[1]Department of Nephrology, Radboud University Nijmegen Medical Center, Nijmegen,
[2]Department of Internal Medicine, Amphia Hospital, Breda,
The Netherlands

Nephrol Dial Transplant 2004; 19(8): 2036-2043

Abstract

Background. A single course of immunosuppressive treatment improves renal survival in patients with idiopathic membranous nephropathy (iMN) and renal insufficiency. However, not all patients respond and relapses occur within 5 years in 30% of patients. It is unknown if a second course of immunosuppressive therapy is effective in such patients.

Methods. We have prospectively studied and evaluated the clinical course in 15 patients (14 male, one female; age 52 ±12 years) with iMN who have received a repeated course of immunosuppressive therapy because of deteriorating renal function associated with relapsing or persistent nephrotic syndrome.

Results. The first course of immunosuppression was started 8 months (range: 0-143 months) after renal biopsy and consisted of chlorambucil (*n*=8) or cyclophosphamide (*n*=7); the second course consisted of cyclophosphamide in all patients. The interval between the first and second course was 40 months (range: 7-112 months). Total follow-up was 110 months (range: 46 –289 months). Renal function and proteinuria improved at least temporarily in all patients after the second course. During follow-up, an additional course of therapy was given in four patients. Status at the end of follow-up was complete remission (*n*=2), partial remission (*n*=8), persistent proteinuria (*n*=3), end-stage renal disease (*n*=1) and death (*n*=1, due to cardiovascular disease while nephrotic). Renal survival was 86% at 5 and 10 years of follow-up. The repeated courses of immunosuppression have resulted in a gain of dialysis-free survival time of $\geq$ 93 months (range: 43-192 months).

Conclusions. Our results indicate that patients with iMN who do not respond well or relapse after a first course of immunosuppressive therapy and have renal insufficiency should be offered a second course of immunosuppression. Such a strategy maintains renal function in the majority of patients.

Introduction

Idiopathic membranous nephropathy is the most common cause of the nephrotic syndrome in adults.[1] Studies on the natural history of the disease show that ≤ 50% of untreated patients will progress to end-stage renal disease (ESRD).[2] A randomized controlled study has provided evidence that a 6 month course of alternating prednisone and chlorambucil improves renal survival in patients with idiopathic membranous nephropathy, a nephrotic syndrome, and normal renal function at the start of therapy.[3] In view of the toxicity of immunosuppressive therapy, most nephrologists have argued that treatment should be restricted to patients with evidence of renal insufficiency or longstanding, severe nephrotic syndrome.[4] Observational studies have, indeed, suggested that immunosuppressive therapy is effective when started in patients with renal insufficiency.[5-8] Most treatment regimens consisted of a combination of prednisone and an alkylating agent, notably chlorambucil or cyclophosphamide, administered for a period of 6-12 months.

Since 1986 we have also used a restrictive treatment policy in patients with idiopathic membranous nephropathy.

We have initially used the so-called Ponticelli regimen, a 6 month course of alternating cycles of prednisone and chlorambucil.[3] From 1991 onwards, we have regularly used a cyclophosphamide-based regimen. Although a single course of immunosuppressive therapy was effective in most patients, some did not respond.[8;9] Furthermore, in ≤ 30% of patients the disease relapsed with recurrent proteinuria.[8;10-12] It is uncertain if a repeated course of immunosuppression is effective in patients with idiopathic membranous nephropathy and persistent or relapsing nephrotic syndrome. We have regularly offered a second course of immunosuppressive therapy to such patients if there was evidence of deteriorating renal function. For the present study we have analysed the outcome in 15 prospectively followed patients who have received a second course of immunosuppressive therapy. Our data indicate that retreatment is effective and attenuates progressive renal failure.

Subjects and Methods

From 1986 onwards we have used immunosuppressive therapy in adult patients with membranous nephropathy. Treatment was restricted to patients with renal insufficiency

(defined as a serum creatinine > 135 μmol/l, a calculated endogenous creatinine clearance < 70 ml/min or a rise in serum creatinine of > 50%) and a proteinuria of ≥ 2.0 g/10 mmol creatinine or to patients with a severe, intolerable nephrotic syndrome. Eligible patients were treated in our University Hospital or by nephrologists in referring hospitals. Details of our treatment protocols and of the outcome in treated patients have been described previously.[5;8;9] In brief, during the period 1986-1991, our immunosuppressive therapy consisted of a 6 month course of alternating cycles of prednisone (1 g methylprednisolone intravenously (i.v.) on three consecutive days, followed by oral prednisone 0.5 mg/kg/day at months 1, 3 and 5) and chlorambucil (0.15 mg/kg/day at months 2, 4 and 6). Thereafter, a regimen containing cyclophosphamide became our therapy of choice. This regimen consisted of corticosteroids (1 g methylprednisolone i.v. on three consecutive days at months 0, 2 and 4, followed by oral prednisone 0.5 mg/kg on alternate days for 6 months) and oral cyclophosphamide in a dose of 1.5-2.0 mg/kg/day for 12 months. All treated patients have been followed prospectively and were regularly seen in the outpatient clinic. For the present study, we have selected all patients who have received a second course of immunosuppressive therapy because of relapsing or persistent nephrotic syndrome and deterioration of renal function.

Calculations and Statistics

For this study, the time of follow-up started at the time of biopsy. Follow-up ended with the last clinical visit or at the occurrence of ESRD or death. The interval between consecutive courses of immunosuppression was calculated from the beginning of the first course to the beginning of the second course. Mean arterial pressure (MAP) was calculated as diastolic blood pressure plus one-third of the pulse pressure (systolic blood pressure - diastolic blood pressure). Proteinuria was expressed as g/10 mmol creatinine (protein-creatinine index). A complete remission of proteinuria (CR), partial remission (PR), persistent proteinuria (PP) 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 case of remission, renal function should have improved or at least stabilized compared with the value at the start of the immunosuppressive therapy. Relapses of proteinuria were defined as nephrotic range proteinuria after a PR or CR of the proteinuria or a rise in proteinuria of > 50% in patients in whom proteinuria had improved initially by > 50%, without reaching values ≤2.0 g/10 mmol creatinine.

For each individual we have estimated dialysis-free survival time gained by therapy. To this end we have plotted the course of 1000/serum creatinine (= glomerular filtration rate (GFR)) *vs* time for each individual patient. The decrease in GFR in the period before the start of immunosuppression was defined by the trend line and the estimated time of onset of ESRD was derived by extrapolation. A value of 1000/serum creatinine of 1 was used as the ESRD reference value. Next, we estimated the expected time of onset of ESRD after treatment by extrapolating a trend line drawn through the point of the last observation in parallel with the first line. The procedure is illustrated for one patient in Figure 1. It is evident that this procedure provides a minimum estimate of the gained dialysis-free survival time, since in most patients there is no apparent decrease in GFR at the moment of last observation.

When considering concomitant drug treatment, special attention was given to the use of angiotensin-converting enzyme inhibitors (ACEI) or angiotensin receptor blockers (AIIA), because of their favourable effects on blood pressure and protein excretion.

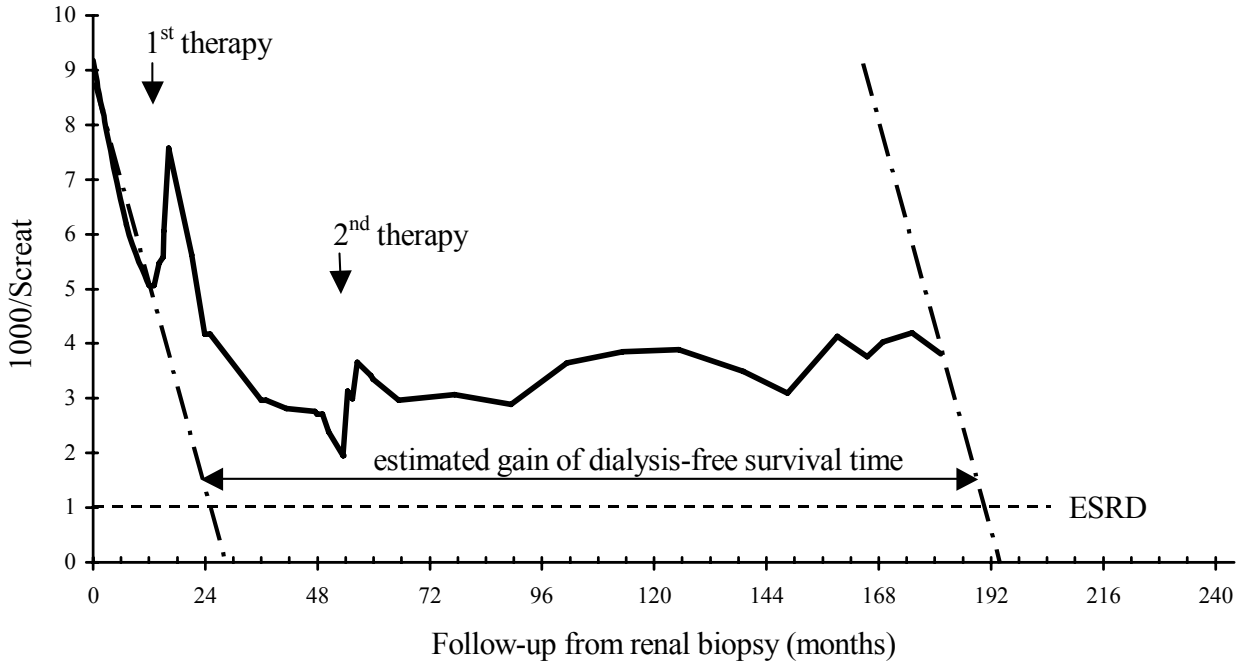


Figure 1. Estimate of dialysis-free survival time gained by therapy.

To estimate the gain of dialysis-free survival time, we have plotted 1000/serum creatinine (1000/Screat; Screat in µmol/l) *vs* time. A trend line was drawn to estimate the decline in renal function before the start of the first immunosuppressive therapy. The expected date of onset of ESRD (without therapy) was derived by extrapolating the trend line. A value of 1000/Screat of 1 was used as a marker of ESRD. The date of onset of ESRD after therapy was estimated from a parallel line, drawn through the point of last observation. The figure represents data from patient no. 3; in this patient the onset of ESRD was delayed by ≥164 months.

Renal survival, defined as being alive without dialysis, was calculated from the time of renal biopsy. The cumulative probabilities of a clinical event (death or ESRD) were estimated according to Kaplan and Meier. Unless otherwise stated, values are given as medians with the range. The Wilcoxon signed rank test was used for comparisons of clinical data within the total group of treated patients at different time points. A *P*-value of < 0.05 was considered significant. All statistical procedures were done using SPSS software (SPSS version 10.0, Chicago, IL, USA).

Results

Fifteen patients (14 male, one female) were eligible for the study. Mean age at biopsy was 51 years (range: 24-68 years). An overview of patient and treatment characteristics, laboratory values at biopsy, time intervals to first immunosuppressive course and between first and second course, and total time of follow-up are given in Table 1.

In Table 2 we have provided detailed information for each patient on the given courses of immunosuppression, side effects, necessary dose reductions, concomitant use of ACEI and/or AIIA, cumulative dosage of cyclophosphamide and estimated gained dialysis-free survival time. Four patients had received prednisone monotherapy in the early disease period. The first course of effective immunosuppression was started at a median of 8 months (range: 0 – 143 months) after renal biopsy and consisted of chlorambucil in eight patients and of cyclophosphamide in seven patients. The interval between the first and second course was 40 months (range: 7 –112 months). All patients received cyclophosphamide as the second course. In three patients (all treated with chlorambucil), retreatment started within 6 months after the end of the first course. In all other patients, retreatment started > 2 years after the end of the first course. Total follow-up was 110 months (range: 46 – 289 months). One patient was lost to follow-up due to non-compliance.

Table 1. Overview of demographic parameters, laboratory values at biopsy, time intervals to first immunosuppressive course and between first and second courses, and total time of follow-up

Patient no.	Sex	Age at biopsy (years)	Screat at biopsy	Proteinuria at biopsy	Interval Bx –1st Th (months)	Interval 1st – 2nd Th (months)	Total FU from biopsy (months)
1	Male	54	104	6.5	45	66	257
2	Male	24	307	5.0	120	38	289
3	Male	56	109	6.7	13	40	181
4	Male	46	83	3.9	15	112	165
5	Male	42	85	15.0	1	35	130
6	Male	66	110	5.7	6	7	133
7	Male	66	103	7.0	8	59	110
8	Male	51	68	6.5	6	74	96
9	Male	45	75	15.9	21	10	99
10	Female	55	117	4.3	143	56	214
11	Male	59	151	7.7	7	12	72
12	Male	48	90	13.4	8	35	60
13	Male	31	125	9.8	16	40	65
14	Male	68	270	6.8	3	36	50
15	Male	39	272	8.9	0	42	46
Median (range)		51 (24-68)	109 (68-307)	6.8 (3.9-15.9)	8 (0-143)	40 (7-112)	110 (46-289)

Screat, serum creatinine (μmol/l) and proteinuria (g/10 mmol creatinine) at the time of renal biopsy; interval Bx–1st Th, time interval between renal biopsy and beginning of first course of effective immunosuppressive therapy; interval 1st –2nd Th, time interval between beginning of first and beginning of second course of effective immunosuppressive therapy; total FU from biopsy, total follow-up time from time of biopsy.

In Table 3 an overview is given of the course of serum creatinine and proteinuria during the two consecutive immunosuppressive courses. We have depicted serum creatinine and urinary protein excretion at the start of the first and second courses of therapy, the lowest value after the start of each course of therapy and the value at the end of follow-up.

Table 2. Overview of given immunosuppression with side effects, use of ACEI and/or AIIA, cumulative dosage of cyclophosphamide and gained time to ESRD

Patient no.	First immunosuppressive therapy			Second immunosuppressive therapy			Cumulative dose of CP	Gained time to ESRD	Remarks
	Agent	Side effects	ACEi/ AIIA	Agent	Side effects	ACEi/ AIIA	(g)	(months)	
1	CA	Leucocytopenia (DR)	No	CP	-	No	57	>192	Third course (CP/aza, 176 months)
2	CA	Leucocytopenia (DR)	Yes	CP	-	Yes	36	>181	
3	CA	Resp. infection (DR)	No	CP	Resp. infection	No	28	>164	
4	CA	Leucocytopenia (DR)	No	CP	Leucocytopenia	Yes	36	>151	
5	CP	-	Yes	CP	Resp. infection(DR)	Yes	89	>124	Third course (CP, 126 months)
6	CA	-	No	CP	-	No	73	>130	Third course (CP, 62 months), fourth course (CsA,102months)
7	CA	Leucocytopenia (DR)	Yes	CP	Leucocytopenia(DR)	Yes	17	>106	
8	CP	-	Yes	CP	-	Yes	146	>93	
9	CA	-	Yes	CP	-	Yes	76	>80	Third course (CP, 27 months)
10	CP	Anemia (DR)	Yes	CP	-	Yes	41	>76	
11	CA	*H. zoster* infection	No	CP	-	No	64	>76	Lost to follow-up
12	CP	Leucocytopenia (DR)	Yes	CP	Leucocytopenia(DR)	Yes	48	>54	
13	CP	Resp. infection (DR)	Yes	CP	-	Yes	28	47	ESRD
14	CP	Leucocytopenia, nausea, oral candidiasis (DR)	Yes	CP	-	Yes	48	>57	
15	CP	Anemia (DR)	No	CP	Leucocytopenia	Yes	68	>48	Died

Patients 1, 2, 10 and 13 received a short course of prednisone monotherapy after renal biopsy.
Agent of immunosuppressive therapy: CA, chlorambucil; CP, cyclophosphamide orally; CP/aza, 3 months cyclophosphamide and 9 months azathioprine; CsA, cyclosporin A. DR, dose reduction because of side effect. Resp. infection, respiratory infection.

Table 3. Efficacy of immunosuppressive courses and status at the end of follow-up

Patient. no.	First Treatment				Second Treatment				Status End FU	
	Screat		Proteinuria		Screat		Proteinuria		Screat	Proteinuria
	Start	Min.	Start	Min.	Start	Min.	Start	Min.		
1	126	126	7.6	0.7	446	174	11.6	1.2	428	1.2
2	408	211	9.6	5.4	313	201	8.7	0.3	228	0.5
3	197	132	7.0	4.0	512	238	10.2	0.4	262	1.2
4	161	82	15.8	0.4	171	132	3.9	0.5	157	0.7
5	85	64	15.0	0.4	162	104	6.6	0.3	87	2.5
6	182	135	10.2	9.4	182	101	9.4	0.7	139	0
7	165	100	6.8	0	158	114	9.8	0.3	127	0.7
8	106	91	4.5	0.9	232	91	5.6	1.3	93	1.3
9	123	88	12.8	4.5	117	83	11.2	4.0	111	0.5
10	171	110	6.0	1.5	266	186	11.1	2.2	186	2.3
11	269	146	7.7	4.4	319	143	18.5	0	143	0
12	184	130	19.1	2.6	208	160	10.1	2.0	194	2.5
13	492	210	19.3	2.9	567	517	2.9	2.4	1000	2.4
14	353	186	5.3	0.5	265	163	9.3	0.8	163	0.8
15	315	135	8.9	1.5	516	235	7.3	6.9	309	6.9
Median	182	130[a]	8.9	1.5[a]	265	160[a]	9.4	0.8[a]	163	1.2
Range	85-492	64-211	4.5-19.3	0-9.4	117-567	83-517	2.9-18.5	0-6.9	87-1000	0-6.9

Screat, serum creatinine (μmol/l); Start, start of the immunosuppressive course; Min., lowest value attained after the respective course; Status End FU, status at the end of total follow-up.
Proteinuria is measured in g/10 mmol creatinine.
[a] Minimum value statistically lower than value at start of therapy.

Efficacy of the first course of immunosuppression

Data are presented in Table 3. Renal function improved in all but one patient, who showed stabilization of serum creatinine. Proteinuria improved in all patients, but CR (one) or PR (seven) was achieved in only eight patients.

Nine out of the 15 patients used an ACEI and/or AIIA at the start of the first course of immunosuppression. Mean arterial blood pressure amounted 103 mm Hg (range: 97-123 mm Hg) at the start of the first immunosuppressive course and 101 mm Hg (range: 87-121 mm Hg) at 12 months (NS).

Efficacy of the second course of immunosuppression

Data are presented in Table 3. At the start of the second course of immunosuppression, renal function was worse in 11 patients in comparison with renal function at the start of the first course and was, by definition, worse in all cases in comparison with the best renal function achieved after the first course. Renal function and proteinuria improved in all patients during the second course of immunosuppression. Ten patients achieved CR (one) or PR (nine). The efficacy of the repeated courses of immunosuppression is visualized in Figures 2 and 3, depicting the time course of the change in 1000/serum creatinine and proteinuria in relation to the start of the second course of therapy. Eleven patients used an ACEI and/or AIIA at the start of the second course of immunosuppression. Despite this, all but one patient had a nephrotic syndrome at this time point. Mean arterial blood pressure was 108 mm Hg (range: 78-130 mm Hg) at the start of the second immunosuppressive course and 95 mm Hg (range: 80-110 mm Hg) at 12 months (NS).

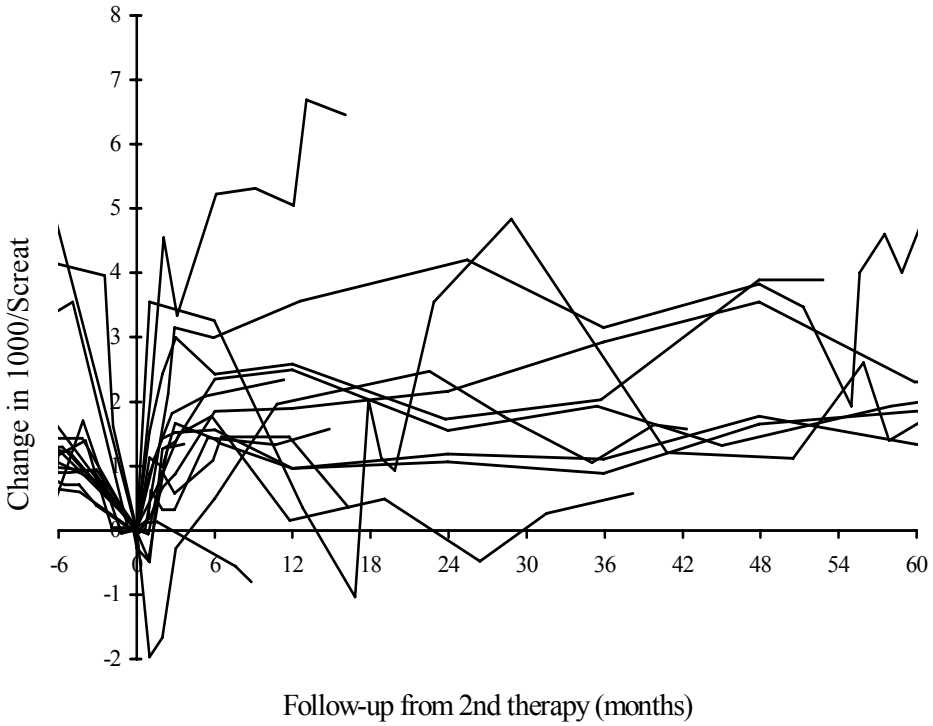


Figure 2. Efficacy of the second course of immunosuppressive therapy on change in renal function.

The start of the second immunosuppressive course is indicated by $t = 0$. Follow-up is shown from 6 months before to 60 months after the second course. The figure plots the change in 1000/serum creatinine value (μmol/l) compared with the value at the start of therapy, which was set at 0.

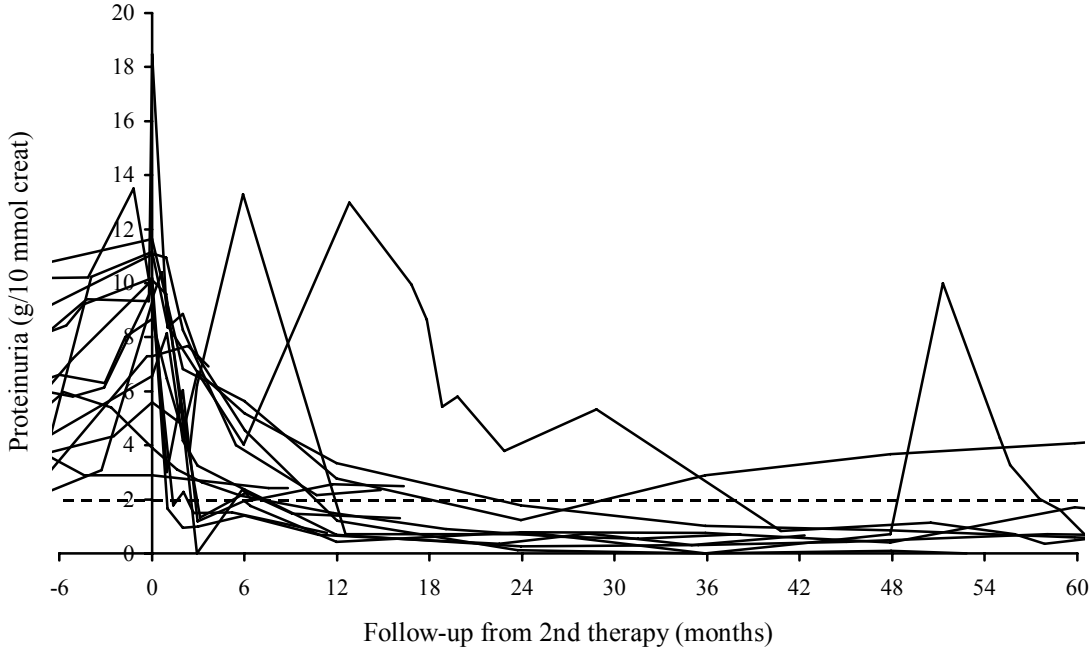


Figure 3. Efficacy of the second course of immunosuppressive therapy on proteinuria.

The start of the second immunosuppressive course is indicated by $t = 0$. Follow-up is shown from 6 months before to 60 months after the second course. Proteinuria is expressed as g/10 mmol creatinine. The reference line reflects a proteinuria of 2 g/10 mmol creatinine, the threshold for defining a partial remission.

Follow-up after the second course

Thus far, four patients have received a third (four) or even fourth (one) course of immunosuppression, because of new relapse (Table 2). At the end of follow-up, two patients were in CR, eight were in PR, three had persistent proteinuria, one had developed ESRD and one had died due to cardiovascular disease while nephrotic (Table 2). Renal survival (alive without dialysis) from the time of biopsy amounted 86% at 5 and 10 years. The repeated courses of immunosuppression have resulted in an estimated dialysis-free survival time gained by therapy of $\geq$ 93 months (range: 43-192 months). This estimate is a minimum and underestimates the real gain, since in seven patients renal function was stable or even improving at the end of follow-up.

Immunosuppressive therapy-related complications

The side effects of immunosuppressive treatment are detailed per patient in Table 2. Side effects were frequent and often necessitated dose reduction. The second course of immunosuppression was not more frequently associated with side effects.

The cumulative dosage of cyclophosphamide was 48 g (range: 17-146 g) (Table 2). In only one patient did the dosage exceed 100 g. This particular patient received a high dose because of his body weight (> 100 kg) and the absence of side effects. The cumulative dose is lower than expected, even in patients who received two courses of cyclophosphamide. This is explained by the fact that, in most patients, dose reduction was necessary because of haematological side effects. Furthermore, if the cumulative dose of cyclophosphamide tended to reach the 100 g threshold, we have favoured to replace cyclophosphamide by azathioprine or cyclosporine (third courses; Table 2).

Discussion

Our study indicates that a second course of immunosuppressive therapy is effective in patients with membranous nephropathy and deteriorating renal function who have not responded well or who have relapsed after a first course of immunosuppressive therapy. In the short-term, renal function improved and proteinuria decreased in all patients during the second course. Also, at the end of follow-up, renal function was maintained in the majority of patients. Admittedly, one-quarter of patients have needed an additional course of treatment. The overall good outcome is reflected in the renal survival rate of 86% at 5 and 10 years from renal biopsy.

Most patients with membranous nephropathy and renal insufficiency will progress to ESRD if left untreated.[7;8;13] Historical control studies have shown renal survival rates of only 20-32% after 7 years.[7;8;13] We and others have provided evidence that a single course of 6-12 months of immunosuppressive therapy is effective and improves renal survival.[7;8;13]

However, with prolonged follow-up it has become evident that many patients will relapse, with a relapse rate of 28% at 5 years follow-up[8]. It is evident that relapses still can occur after a second course of immunosuppressive therapy (in our present study, four out of 15 patients have needed a third course of immunosuppression).

Our study, thus, provides arguments for an active treatment policy in patients with membranous nephropathy, relapsing proteinuria and deterioration of renal function. Although our study cohort is relatively small, to date there are few data on this particular group of patients available in the literature.[13;14] Most data on repeated courses of immunosuppressive therapy relate to patients with relapsing nephrotic syndrome without renal insufficiency.[3;10;14;15] Ponticelli *et al.*[10] reported on 169 patients with a variable degree of proteinuria and normal renal function, half of whom were treated with immunosuppression. Of 111 patients entering a PR or CR, 42 relapsed to nephrotic range proteinuria. Relapses occurred more frequently after a PR. Half of the relapsing patients entered a spontaneous remission again. Fifteen patients were treated again; nine of 11 patients treated with chlorambucil had a persistent remission on retreatment. Repeated cytotoxic therapy clearly increased the chance of a stable remission. Renal function deterioration occurred in a minority of patients (six out of 42), mainly in patients with persistent proteinuria.[10] Positive responses to retreatment in low-risk populations have been reported by others as well.[14;15] However, relapses after retreatment occur even in this low-risk population.[3;10;15]

Blood pressure control in the early years of the study was not optimal when considering current guidelines. Treatment of blood pressure has become more aggressive in the recent decade. This is reflected by the fact that MAP amounted 103 mm Hg at the start of the first course and 95 mm Hg at 12 months after the second course of immunosuppressive therapy. The target MAP of 92 mm Hg is difficult to achieve in patients with renal failure and older age. However, most patients were using ACEI or AIIA and it is unlikely that a more aggressive antihypertensive strategy could have prevented the observed deterioration of renal function, thus, obviating the need for repeated immunosuppressive therapy. It is well established that renal function may deteriorate in many patients with membranous nephropathy despite well-controlled blood pressures and the use of ACEI or AIIA.[8]

Two important questions must be addressed with respect to our treatment strategy. First, is it allowed to delay the start of immunosuppressive therapy until renal dysfunction is apparent? There is no support for this strategy from randomized trials. However, a recent analysis of our data has provided arguments that a restrictive treatment policy is justified. We have studied the outcome of a restrictive treatment strategy in a large cohort of adult patients with membranous nephropathy. Details of this study will be published elsewhere.[16] Thus far, nearly half of the patients have received immunosuppressive therapy, mainly because of renal insufficiency. At the end of follow-up, 67% of patients were in CR or PR. Renal survival was

94% at 5 years and 88% at 7 years. From these data we concluded that restricted therapy is justified in view of the good overall outcome, whilst preventing immunosuppressive therapy in more than half of the patients.

The second question is whether the advantages of a second (or even third or fourth) course of immunosuppressive therapy outweigh the short- and long-term side effects, particularly in patients with established moderate to severe renal insufficiency. To be able to balance the benefits and risks, we have estimated the dialysis-free survival time gained by therapy. It is evident that treatment-attributable survival time greatly exceeds the duration of the treatment courses. Furthermore, in the discussion of the side effects of treatment, it is important to realize that most patients who develop ESRD will receive a kidney transplant, thus, necessitating life-long immunosuppression with the related side effects. Our data indicate that the use of a second course of immunosuppressive therapy is not associated with more frequent or more severe side effects in the short-term. Of course, it is important to consider the long-term side effects, the most important one being the potential of cyclophosphamide to induce (bladder) malignancies.[17;18] For this reason, we have often replaced cyclophosphamide by azathioprine or cyclosporine whenever a third or fourth course of immunosuppressive therapy was needed. The risk of cyclophosphamide-related bladder cancer increases with the duration (especially > 2.7 years) and the cumulative dosage (mainly > 100g) of cyclophosphamide treatment.[17] The cumulative duration of cyclophosphamide therapy and the administered dosage of cyclophosphamide are well below these values in most of our patients.

It is evident that not all patients respond well to treatment and it is likely that non responsive patients may develop ESRD with longer follow-up. One could consider taking a renal biopsy to aid in treatment decisions, particularly to prevent that treatment is instituted in patients with chronic sclerotic lesions.

It might be questioned whether it is possible to reduce the dose of cyclophosphamide. Ponticelli *et al.*[11] have used cyclophosphamide in an alternating schedule, limiting the duration of cyclophosphamide therapy to 3 months. However, this short regimen has been studied extensively only in patients with normal renal function. We previously have reported a comparison of the Ponticelli regimen (with chlorambucil) and our 12 month cyclophosphamide regimen.[5] We found the cyclophosphamide regimen more effective and less toxic than chlorambucil. It remains to be proved if these differences are related to the difference in duration of treatment or the type of agent. Future studies are needed to settle this issue; meanwhile we favour the 12 month regimen in patients with renal insufficiency.

Another strategy would be to use other immunosuppressive agents, such as mycophenolate mofetil or cyclosporine. To date, information on the efficacy of mycophenolate mofetil in patients with idiopathic membranous nephropathy and renal insufficiency is limited and inconclusive. Cyclosporine has been evaluated in a small randomized study, demonstrating an improvement in creatinine clearance and a decrease in proteinuria.[19] Relapses occur, however, in about one-third of patients after cyclosporine withdrawal, thus, necessitating long-term administration of the drug.[20;21] Furthermore, transient renal dysfunction and hypertension can occur in cyclosporine-treated patients. [19-21]

In conclusion, a repeated course of immunosuppressive therapy with cyclophosphamide and prednisone improves renal function and retards the progression of renal insufficiency in patients with idiopathic membranous nephropathy, relapsing proteinuria and deteriorating renal function. The advantage of a renewed course of immunosuppressive treatment (i.e. a delay of onset of ESRD) must be weighed against the side effects. We would like to argue in favour of a strategy of repeated immunosuppression, in particular in patients who would otherwise progress to ESRD, necessitating renal transplantation with its associated life-long immunosuppression.

References

1. Haas M, Meehan SM, Karrison TG, Spargo BH. Changing etiologies of unexplained adult nephrotic syndrome: a comparison of renal biopsy findings from 1976-1979 and 1995-1997. *Am J Kidney Dis* 1997; 30: 621-631
2. Marx BE, Marx M. Prognosis of idiopathic membranous nephropathy: a methodologic meta-analysis. *Kidney Int* 1997; 51: 873-879
3. Ponticelli C, Zucchelli P, Passerini P, *et al*. A 10-year follow-up of a randomized study with methylprednisolone and chlorambucil in membranous nephropathy. *Kidney Int* 1995; 48: 1600-1604
4. Cattran DC. Idiopathic membranous glomerulonephritis. *Kidney Int* 2001; 59: 1983-1994
5. Branten AJ, Reichert LJ, Koene RA, Wetzels JF. Oral cyclophosphamide versus chlorambucil in the treatment of patients with membranous nephropathy and renal insufficiency. *Q J Med* 1998; 91: 359-366
6. Stirling CM, Simpson K, Boulton-Jones JM. Immunosuppression and outcome in idiopathic membranous nephropathy. *Q J Med* 1998; 91: 159-164
7. Torres A, Dominguez-Gil B, Carreno A, *et al*. Conservative versus immunosuppressive treatment of patients with idiopathic membranous nephropathy. *Kidney Int* 2002; 61: 219-227

8. Du Buf-Vereijken PW, Branten AJ, Wetzels JF. Cytotoxic therapy for membranous nephropathy and renal insufficiency: improved renal survival but high relapse rate. *Nephrol Dial Transplant* 2004; 19: 1142-1148

9. Branten AJ, Wetzels JF. Short- and long-term efficacy of oral cyclophosphamide and steroids in patients with membranous nephropathy and renal insufficiency. *Clin Nephrol* 2001; 56: 1-9

10. Ponticelli C, Passerini P, Altieri P, *et al.* Remissions and relapses in idiopathic membranous nephropathy. *Nephrol Dial Transplant* 1992; 7 [Suppl 1]: 85-90

11. Ponticelli C, Altieri P, Scolari F, *et al*. A randomized study comparing methylprednisolone plus chlorambucil versus methylprednisolone plus cyclophosphamide in idiopathic membranous nephropathy. *J Am Soc Nephrol* 1998; 9: 444-450

12. Laluck BJ, Jr, Cattran DC. Prognosis after a complete remission in adult patients with idiopathic membranous nephropathy. *Am J Kidney Dis* 1999; 33: 1026-1032

13. Jindal K, West M, Bear R, Goldstein M. Long-term benefits of therapy with cyclophosphamide and prednisone in patients with membranous glomerulonephritis and impaired renal function. *Am J Kidney Dis* 1992; 19: 61-67

14. Faedda R, Satta A, Bosincu L, *et al.* Immunosuppressive treatment of membranous glomerulonephritis. *J Nephrol* 1995; 8: 107-112

15. Suki WN, Trimarchi H, Frommer JP. Relapsing membranous nephropathy. Response to therapy of relapses compared to that of the original disease. *Am J Nephrol* 1999; 19: 474-479

16. Du Buf - Vereijken PW, Feith GW, Hollander D, *et al.* Restrictive use of immunosuppressive treatment in patients with idiopathic membranous nephropathy: high renal survival in a large patient cohort. *Q J Med* 2004; 97: 353-360

17. Talar-Williams C, Hijazi YM, Walther MM, *et al*. Cyclophosphamide-induced cystitis and bladder cancer in patients with Wegener granulomatosis. *Ann Intern Med* 1996; 124: 477-484

18. Knight A, Askling J, Ekbom A. Cancer incidence in a population-based cohort of patients with Wegener's granulomatosis. *Int J Cancer* 2002; 100: 82-85

19. Cattran DC, Greenwood C, Ritchie S, *et al*. A controlled trial of cyclosporine in patients with progressive membranous nephropathy. Canadian Glomerulonephritis Study Group. *Kidney Int* 1995; 47: 1130-1135

20. Rostoker G, Belghiti D, Ben Maadi A, *et al*. Long-term cyclosporin A therapy for severe idiopathic membranous nephropathy. *Nephron* 1993; 63: 335-341

21. Fritsche L, Budde K, Farber L, *et al*. Treatment of membranous glomerulopathy with cyclosporin A: how much patience is required? *Nephrol Dial Transplant* 1999; 14: 1036-1038

Chapter 4

Mycophenolate mofetil versus cyclophosphamide in patients with idiopathic membranous nephropathy and renal insufficiency

Peggy W.G. du Buf-Vereijken[1,2] and Jack F.M. Wetzels[2]

[1]Department of Internal Medicine, Amphia Hospital, Breda,
[2]Department of Nephrology, Radboud University Nijmegen Medical Centre, Nijmegen,
The Netherlands

Abstract

Background. Patients with idiopathic membranous nephropathy (iMN), a nephrotic syndrome and renal insufficiency are at high risk for progression to ESRD. We have demonstrated that treatment with cyclophosphamide (CP, 1.5-2.0 mg/kg/day for 12 months) and steroids is effective, but associated with frequent and severe side effects.

Methods. From May 2002 we have used a treatment schedule consisting of mycophenolate mofetil (MMF, 1000 mg twice daily for 12 months) and steroids. Efficacy and side effects of this schedule are compared with the results of treatment with CP in historical control patients, matched for serum creatinine, proteinuria, age and previous immunosuppressive treatment.

Results. Thirteen patients (9 M, 4 F; median age 53 years) have completed the treatment protocol with MMF. For comparison we have selected 13 patients treated with CP. All patients but one (CP-group) used an ACE-inhibitor and/or an angiotensin receptor blocker. Blood pressures were comparable. Treatment with MMF was as effective as CP in improving renal function: serum creatinine at start of therapy and at 12 months was 162 [98-378] and 123 [90-454] µmol/l (MMF) *vs* 158 [117-386] and 113 [88-289] µmol/l (CP)(NS). MMF was as effective as CP in reducing proteinuria: proteinuria at start and at 12 months was 13.2 [3.6-30.8] and 2.0 [0.0-12.2] g/day (MMF) *vs* 11.6 [4.3-23.0] and 0.9 [0.1-13.0] g/day (CP)(NS). Eight (MMF) resp. 7 (CP) patients developed a partial remission of proteinuria. Side effects were reported in 9 out of 13 patients treated with MMF (69%), and 10 out of 13 (77%) treated with CP (NS). In the MMF-treated patients side effects were less severe, and necessitated dose reduction or interruption of therapy in 3 patients as compared with 9 patients treated with CP ($P < 0.05$) Treatment failure occurred in two MMF- and none of the CP-treated patients.

Conclusion. MMF was better tolerated than CP. In the short-term MMF is as effective as CP in improving renal function, reducing proteinuria and achieving an initial remission of proteinuria in patients with idiopathic membranous nephropathy and renal insufficiency. Thus far, treatment failure occurred in two patients in the MMF-treated group en in none in the CP-treated group. A larger group of patients and longer follow-up is needed to confirm the long-term efficacy of MMF therapy.

Introduction

Idiopathic membranous nephropathy remains the most common cause of the nephrotic syndrome in adults.[1] Studies on the natural history of the disease show that up to 40% of untreated patients will progress to end-stage renal disease (ESRD).[2-5] We, and others, are in favour of restricting immunosuppressive therapy to patients at highest risk to develop ESRD.[5-13] Besides a sustained high amount of proteinuria, deterioration of renal function is an important prognostic factor predicting chronic renal insufficiency in this patient group.[14-16] We have demonstrated that treatment with cyclophosphamide (1.5-2.0 mg/kg/day for 12 months) and steroids (methylprednisolone 1 g i.v. on 3 consecutive days on months 0, 2 and 4 and prednisone 0.5 mg/kg/48 hours for 6 months) in patients with idiopathic membranous nephropathy and renal failure is effective, with a remission rate of 86%.[8] Renal prognosis improved from a 7 year renal survival of 32% in untreated historical controls to 74% in cyclophosphamide treated patients.[8] A comparable improvement in renal survival was shown for similar high-risk patients treated with chlorambucil.[12]

However, treatment with cyclophosphamide or chlorambucil is associated with frequent and severe side effects, especially in patients with renal insufficiency.[7;8] Mycophenolate mofetil is a new immunosuppressive agent, with fewer side effects and proven efficacy in transplant patients. Therefore, we have started a pilot-study to evaluate mycophenolate mofetil as treatment agent in patients with idiopathic membranous nephropathy, a nephrotic syndrome and renal insufficiency. We have compared the efficacy and side effects with results obtained in historical control patients treated with cyclophosphamide.

Subjects and Methods

In our pilot study with MMF we included only adult patients (age > 18 years) with a biopsy proven membranous nephropathy. A secondary cause of membranous nephropathy was excluded on clinical and/or laboratory grounds. Patients were recruited in our University Hospital or in one of 9 referring hospitals. Eligible patients had to have evidence of renal insufficiency (defined as a serum creatinine > 135 μmol/l, a calculated endogenous creatinine clearance < 70 ml/min or a rise in serum creatinine of > 50%) and a proteinuria of at least 2.0 g/10 mmol creatinine. Exclusion criteria were systemic diseases, malignancies, active

infection, pregnancy or inadequate contra-conception, unstable angina pectoris, diabetes mellitus type I or long-lasting diabetes mellitus type II (unless renal biopsy proved the absence of diabetic nephropathy), clinical evidence of renal vein thrombosis, liver test abnormalities (> 2x upper limit of normal), active peptic ulcer disease or gastro-intestinal diseases that could impair the resorption of oral medication. Patients who had used immunosuppressive therapy in the previous six months were not eligible, except in case of evident treatment failure. These inclusion and exclusion criteria also were used to determine the eligibility of patients for treatment with cyclophosphamide in the period 1995-2002.

Treatment consisted of methylprednisolone 1 g i.v. for three consecutive days at the beginning of months 0, 2 and 4, and oral prednisone 0.5 mg/kg every other day for 6 months with subsequent tapering. The patients treated with MMF were treated with oral MMF 1000 mg twice daily for 12 months. In case of anemia (Hb < 6.0 mmol/l) or diarrhoea, the dose was initially reduced with 50% and subsequently up titrated to the highest tolerated dose. Historical controls were treated with oral cyclophosphamide, 1.5-2.0 mg/kg/day for 12 months, with dose reductions in case of side effects.[8] All patients were advised a moderately salt restricted diet. Concomitant treatment was not standardized, however physicians were instructed to lower blood pressure aggressively, primarily by using ACE-inhibitors and/or angiotensin receptor blockers for all patients with proteinuria, in the maximum tolerated dose, and to titrate on blood pressure (target mean arterial pressure (MAP) 92 mm Hg) and proteinuria. HMG-CoA reductase inhibitors were used to lower serum cholesterol. Anticoagulant drugs were not routinely prescribed. For the prevention of gastric complaints, famotidine was added. We advised to add trimethoprim-sulfamethoxazole 480 mg daily in the first 4-6 months in patients treated with cyclophosphamide to prevent *Pneumocystis carinii* pneumonia. Such preventive treatment was not used during MMF treatment.

The time of follow-up started at the beginning of treatment with either MMF or CP. Efficacy and side effects of both treatment regimens were compared during the treatment year. Patients were seen at least every 4-8 weeks during the treatment year. Blood pressure, side effects of the therapy and laboratory data were registered.

From our database, we selected 13 patients treated with cyclophosphamide as historic controls for the 13 patients treated with MMF. For matching, we used the following criteria in descending order of relevance: serum creatinine and proteinuria at the start of therapy, age, previous immunosuppressive therapy, serum albumin at start of therapy and sex.

In most MMF-treated patients, the urinary excretion of IgG, β_2-microglobulin and α_1-microglobulin were measured at 0, 2, 6 and 12 months in a standardized way.[17] These parameters of glomerular perm selectivity and tubular proteinuria respectively, are well known prognostic markers in patients with idiopathic membranous nephropathy and normal renal function.[17-20] We previously have validated the following threshold values: for IgG 250 mg/24 hour, for β_2-microglobulin 0.5 μg/min and for α_1-microglobulin 40 μg/min. We have compared the results with data obtained in patients treated with CP. However, since such measurements were only recently introduced data for comparison were not available for all matched CP-treated controls. Therefore, for the comparison data of 11 less well-matched CP-treated patients were used.

Calculations and Statistics

For descriptive statistics, results are given as means ± standard deviation or medians with range when appropriate. To correct for inappropriate 24-hour urine collections, the amount of proteinuria was expressed as a protein-creatinine index (g/10 mmol creatinine). 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 case of remission renal function should have improved or at least stabilized. All patients who entered a complete remission were also registered as having a partial remission. A relapse was defined as nephrotic range proteinuria after a partial or complete remission of 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. Treatment failure was defined as a relapse of nephrotic range proteinuria with renal function deterioration, defined as a rise in serum creatinine of at least 25% over the best value achieved in the treatment year.

In case of treatment failure before the end of the treatment year, the last laboratory values before the start of rescue therapy were carried forward to determine the 12 months values. Mean arterial blood pressure (MAP) was calculated using de formula MAP = diastolic blood pressure + 1/3 x (systolic blood pressure - diastolic blood pressure).

The Mann-Whitney test was used for comparison between groups, and the Wilcoxon signed rank test for comparisons within the groups of treated patients. A *P*-value of < 0.05 was considered significant. All statistical procedures were done using SPSS software (SPSS version 11.5, Chicago, IL, USA).

Results

From May 2002 until May 2004 22 patients have been included in the MMF study. Thirteen patients have completed the 12-months treatment protocol or had a treatment failure within the treatment year.

The patient characteristics of the 13 MMF-treated patients and 13 matched, historical control CP-treated patients are given in Table 1. The groups were well matched for previous immunosuppression, sex, age, serum creatinine, serum albumin, proteinuria and MAP at the start of therapy. The time between renal biopsy and the start of the currently evaluated immunosuppressive therapy was shorter in the MMF-treated group ($P < 0.05$). This is explained by the fact that serum creatinine at the time of biopsy was numerically higher in MMF treated patients (130 [76-301] *vs* 98 [75-118] μmol/l), and in 6 exceeded 135 μmol/l, the threshold for starting immunosuppressive therapy. In the MMF-treated group, all patients used ACE-inhibitors and/or angiotensin receptor blockers, in the CP-treated group one patient did not receive such therapy. Six CP-treated individuals received trimethoprim-sulfamethoxazole as *Pneumocystis carinii* pneumonia prophylaxis, whereas, per protocol, none of the MMF-treated patients did.

Table 1. Baseline characteristics

	MMF (*n*=13)	CP (*n*=13)
Previous immunosuppression	Prednisone, *n*=1 Prednisone, CP, *n*=2 CP, *n*=1	Prednisone, *n*=1 Chlorambucil, *n*=1 Chlorambucil, CP, *n*=1 CP, *n*=1
Sex (M:F)	9 : 4	11 : 2
Age at start of study (years)	53 [38-69]	60 [47-78]
Time between renal biopsy and start of study (months)	3* [1-96]	14 [7-77]
Serum creatinine (μmol/l)	162 [98-378]	158 [117-386]
Serum albumin (g/l)	21 [6-36]	22 [14-31]
Proteinuria (g/10 mmol creatinine)	13.2 [3.6-30.8]	11.6 [4.3-23.0]
Mean Arterial Pressure (mm Hg)	99 [76-117]	93 [80-132]

Values are medians with range.
* $P < 0.05$, MMF *vs* CP

In Table 2, serum creatinine, serum albumin, serum cholesterol, proteinuria and MAP at start of therapy, at 6 months and at the end of the treatment are given. Treatment with MMF was as effective as CP in improving renal function and lowering proteinuria. In the MMF-treated group, 7 patients developed an initial partial remission, as compared with 8 patients in the CP-treated group (NS). In both groups one patient achieved a complete remission within the treatment year. Cumulative (partial) remission rate at 12 months was 54%±14% on MMF and 62%±13% on CP. The onset of partial remissions was at a median of 6.4 and 6.8 months, MMF *vs* CP (NS).

Table 2. Laboratory parameters and blood pressure during the treatment-year

	MMF (n=13)			CP (n=13)		
	start	6 months	12 months	start	6 months	12 months
Screatinine (µmol/l)	162 [98-378]	112** [86-268]	123 [90-454]	158 [117-386]	113** [76-356]	113** [88-289]
Salbumin (g/l)	21 [6-36]	32** [21-45]	35** [27-43]	22 [14-31]	34** [22-38]	39** [25-44]
Scholesterol (mmol/l)	7.1 [4.9-15.0]	6.7 [4.1-9.8]	4.7** [3.2-8.6]	7.0 [5.4-9.7]	6.1* [4.7-8.6]	5.2** [3.8-7.5]
Proteinuria (g/10 mmol creat)	13.2 [3.6-30.8]	3.4** [0.0-10.0]	2.0** [0.0-12.2]	11.6 [4.3-23.0]	2.4** [0.2-10.0]	0.9** [0.1-13.0]
MAP (mm Hg)	99 [76-117]	99 [85-113]	95 [75-110]	93 [80-132]	89**,# [75-120]	93 [83-124]

Abbreviations: S, serum; MAP, mean arterial pressure. Values are medians with range.
* $P < 0.05$, ** $P < 0.01$ for comparison with laboratory values at start of therapy.
# $P < 0.05$, for comparison of MMF *vs* CP

In the MMF-treated group, two patients developed a relapse of the nephrotic syndrome at 8 months. One of these had an initial improvement in proteinuria from 13 to 3 g/day, the other experienced a short lasting PR at 7 months. In the CP-treated group no patient relapsed during the treatment year. As both relapsing patients experienced deterioration in renal function, after an initial improvement in renal function on MMF-therapy, they have started rescue therapy with cyclophosphamide and are recorded as treatment failures.

The results at the end of the treatment year are given in Table 3. There are no major differences, although treatment failure only was observed in the MMF group.

Table 3. Status at end follow-up

	MMF (n=13)	CP (n=13)
Complete remission (< 0.2 g/day)	1 (8%)	1 (8%)
Partial remission (0.21-2.0 g/day)	5 (38%)	7 (54%)
Nephrotic syndrome	5 (38%)	5 (38%)
Treatment failure	2 (15%)	0 (0%)

In Table 4, the results of the urinary measurements of IgG, β_2-microglobulin and α_1-microglobulin are shown. Although the treatment groups were comparable with respect to renal function, proteinuria was higher in the MMF-treated group. In both groups a similar decline in the urinary excretions of IgG, β_2-microglobulin and α_1-microglobulin was noted.
At 12 months urinary IgG was below the threshold of 250 mg/24 hours in 8 MMF and all 11 CP-treated patients. The urinary excretion of β_2-microglobulin was below the threshold of 0.5 μg/min in 4 out of 11 MMF-treated and 4 out of 10 CP-treated patients; in one CP-treated patient urinary pH was not > 6.0 at 12 months thus unabling correct determination of urinary β_2-microglobulin.The urinary excretion of α_1-microglobulin was below the threshold of 40 μg/min in 6 MMF- as well as 6 CP-treated patients. Therefore, on the parameters for glomerular permeability and tubular proteinuria, no difference in the efficacy of both treatment agents existed.
In Table 5, side effects in both groups are shown. In the 13 patients treated with MMF, therapy-related side effects occurred in 9 patients (69% of patients): anemia (n=4), respiratory tract infections (n=2; 1 with tuberculosis), steroid diabetes (n=2) and diarrhoea (n=1). Furthermore, one patient with longstanding diabetes mellitus type II without signs of diabetic nephropathy in the renal biopsy, experienced a diabetic foot. Another MMF-treated patient developed severe angio-edema, necessitating even mechanical ventilation, probably related to the use of RAAS-blockers; re-challenge with MMF was uneventful.

Table 4. Comparison of urinary markers of glomerular permeability and tubular proteinuria in 11 patients treated with MMF or CP

	MMF (*n*=11)				CP (*n*=11)			
	start	2 months	6 months	12 months	start	2 months	6 months	12 months
Screatinine (µmol/l)	143 [98-177]	121** [77-156]	112** [81-160]	120** [81-180]	152 [132-278]	138* [108-221]	128** [94-168]	132** [95-215]
$S\beta_2m$ (mg/l)	5.2 [3.2-12.5]	3.4* [1.6-6.5]	3.4** [1.7-5.9]	3.0* [1.5-9.4]	5.3 [3.1-7.5]	3.5** [2.4-5.7]	3.4** [2.2-5.4]	3.5* [1.9-7.2]
Proteinuria (g/10 mmol creat)	12.7# [6.3-28.1]	7.8**,# [0.2-17.8]	4.7**,# 0.1-14.1]	2.0** [0.1-10.8]	8.9 [5.3-12.8]	3.3** [1.2-7.8]	1.8** [0.5-3.4]	0.9** [0.2-2.6]
Urinary markers								
IgG (mg/24 hour)	475 [74-3082]	123** [1-801]	29** [3-229]	43** [8-946]	363 [188-859]	61** [5-253]	19** [4-105]	19** [14-124]
β_2m (n=5 *vs* 6) $ (µg/min)	9.6 [1.3-62.0]	4.3* [0.1-31.6]	0.7* [0.0-12.8]	0.2 [0.1-29.3]	10.8 [2.5-46.9]	2.4 [0.3-56.8]	4.5 [0.4-37.6]	4.1 [0.5-47.3]
α_1m (µg/min)	119 [25-431]	93* [1-267]	33** [2-145]	39** [7-202]	108 [17-307]	56 [17-182]	34* [8-126]	19* [10-138]

Values are median with range.
β_2m = β_2- microglobulin and α_1m = α_1-microglobulin
*P < 0.05, **P < 0.01 for comparison of laboratory values at 2, 6 and 12 months compared with start of therapy, within a treatment group.
# P < 0.05, for comparison of MMF *vs* CP.
$, only those patients with a urinary pH > 6.0 at all time points are included.

In the CP-treated patients, 10 patients experienced side effects, mainly consisting of leucocytopenia (n=7), anemia (n=2) and infections (n=6). One patient developed a pancytopenia with severe sepsis. Two patients experienced general malaise.

In general, side effects were less severe with MMF. This is reflected by the fact that side effects necessitated dose reduction in only 3 MMF patients *vs* 9 CP patients ($P < 0.05$). Most important, leucocytopenia, quite common in CP-treated patients, was not seen in MMF-treated patients. Infections were more frequent with CP-use, compatible with the increased incidence of leucocytopenia, but especially noteworthy in view of the use of trimethoprim-sulfamethoxazole in half of the patients treated with CP.

Table 5. Treatment-related complications

Side effect	MMF (n=13)		CP (n=13)	
	Number of pts (%)	Dose reduction	Number of pts (%)	Dose reduction
Bone marrow depression				
Leucocytopenia	0 (0%)	0*	7 (54%)	7
Anemia	4 (31%)	2	2 (15%)	1
Infections				
All together	2 (15%)	0	6 (46%)	3
Respiratory	2 (15%)	0	3 (23%)	1
Diarrhoea	1 (8%)		0 (0%)	0
Malaise	0 (0%)	0	2 (15%)	0
Steroid induced diabetes mellitus	2 (15%)	0	0 (0%)	0
Other	2 (15%)	0	1 (8%)	1
Total	9 (69%)	3*	10 (77%)	9

The numbers don't add up; one patient can have more than one complication.
Definitions: leucocytopenia: leucocyte count < $3.0*10^9$/l; anemia: Hb < 6.0 mmol/l or erythropoietin use to prevent Hb < 6.0 mmol/l; thrombocytopenia: thrombocyte count < $100*10^9$/l.
* $P < 0.05$, MMF *vs* CP

Discussion

Immunosuppressive therapy is effective in patients with idiopathic membranous nephropathy and renal insufficiency. However, side effects are a major limitation of the use of immunosuppressive agents such as CP or chlorambucil in the treatment of patients with idiopathic membranous nephropathy. Therefore, MMF may offer benefits.

Our data indicate that, in the short-term, MMF is as effective as CP in the treatment of patients with idiopathic membranous nephropathy and renal insufficiency. Of course, since this was only a pilot study in a limited number of patients, firm conclusions cannot be drawn. However, we feel that our data support the conception of controlled studies using MMF.

Literature data on the efficacy of MMF in patients with idiopathic membranous nephropathy are scarce and preliminary.

Briggs described the first results in three patients with membranous nephropathy, a nephrotic syndrome and normal renal function, relapsing after therapy consisting of corticosteroids and cyclosporine.[21] MMF was given in a dose of $\geq$ 750 mg twice daily, initially with low dose steroids, which were subsequently withdrawn. With a short course of MMF, significant reductions in proteinuria were demonstrated to levels below 2g/day, with preservation of normal renal function. Side effects were absent.[21] Follow-up of these patients suggests that relapse may occur after a six month course, with partial remission after the resumption of MMF in one patient.[22] In a later report, the experience was extended into 17 patients with idiopathic membranous nephropathy, 15 of them nephrotic and six with renal insufficiency.[23] Indications for MMF treatment included steroid and/or cyclosporine dependency, resistance or intolerance. There was no predefined treatment protocol. The duration of MMF-therapy was highly variable: 4-25 months and the dosage varied from 0.5 to 1.0 g twice daily, with 10 patients receiving 2.0 g/day. Most patients received low dose steroids. Of the 15 nephrotic patients, two achieved a complete and five a partial remission (remission rate 41%). The median percent reduction in proteinuria was 61%. There was no change in median serum creatinine. Two patients relapsed after MMF was stopped. In the majority of patients, progressive steroid and cyclosporine withdrawal was achieved. Side effects consisted of mild reversible leucocytopenia (n=1) and discontinuation of MMF was needed in three patients due to severe gastritis, pneumonia and squamous cell cancer of the arm. A striking observation in these studies was a threshold dose of 1.5 g/day for efficacy, unless there was severe renal insufficiency.[23]

Miller reported on 16 patients with membranous nephropathy and a nephrotic syndrome, of whom 15, 6 and 5 had failed on therapy with steroids, cytotoxic agents or cyclosporine A, respectively.[24] All patients were at high risk for progressive renal failure, given their persistent high level of proteinuria and eleven patients had renal insufficiency (serum creatinine > 135 μmol/l). The administered dose of MMF was low (500 –2000 mg/day, only 7 patients achieved the target dose of 2 g/day). MMF was given for a mean period of 8 months, and was discontinued in patients with no response at 6 months. Additional low dose steroid therapy (< 15 mg/day) was given in only five patients. Most patients used RAAS-blockers and HMG-CoA reductase inhibitors. Six patients (38%) experienced a halving of proteinuria, which occurred after a mean duration of 6 months of therapy. A partial remission of proteinuria (< 3 g/day) occurred in only two patients; both patients were treated for about one year and with doses of 1.0-1.5 g/day of MMF. No significant change in serum creatinine level was seen. Again, side effects were infrequent and mild with transient leucocytopenia in one patient, *Varicella Zoster* infection in another patient and severe diarrhoea in one patient, necessitating discontinuation of the drug in these last two patients.[24]

Polenakovic described the effect of MMF-treatment in eight patients with membranous nephropathy, in whom previous treatment failed in five patients (previous treatment consisted of steroids with cyclosporine, chlorambucil or cyclophosphamide) and three patients were treated with MMF as first choice drug.[25] All patients were nephrotic, three had slight elevations of serum creatinine. Treatment consisted of MMF 2g/day during nine months. Proteinuria decreased significantly from 4.4 g/day to 1.9 g/day after nine months. Renal function did not improve significantly. Side effects were noted in one patient, who experienced joint and muscle aches.[25]

Our study demonstrates the short-term efficacy of mycophenolate mofetil (MMF) with steroids in a quite homogeneous group of patients with idiopathic membranous nephropathy and renal insufficiency, thus patients at high risk for progressive renal insufficiency. Efficacy of MMF may seem better than expected based on the literature data. We feel that our data are rather robust, since we have been able to compare the efficacy of MMF with the efficacy of cyclophosphamide (CP) and steroids in a group of well-matched historical control patients. Both patient groups were comparable at the start of treatment with respect to those parameters that are most important predictors of renal insufficiency: serum creatinine and amount of proteinuria at the start of treatment. Furthermore, they were comparable on other

characteristics, like previous immunosuppression, age, serum albumin, MAP and the use of RAAS-blockers.

Our results may be explained by the treatment protocol. We have treated almost all patients with MMF 2 g/day for 12 months and (initial high dose) steroids for 6 months in a predefined treatment protocol. Thus, our treatment schedule clearly outweighs the schedules given by other investigators.

In the short-term, MMF was as effective as CP in improving renal function and reducing proteinuria. An equal substantial number of patients developed a remission of proteinuria. Most patients experienced an improvement in renal function, in contrast to literature results hitherto reported.

We must caution however, since we cannot comment on the long-term efficacy. Thus far, two patients have already experienced treatment failure and have needed additional therapy with CP. This might suggest that MMF is less able to induce sustained remissions. We know, from our previous study, that the rate of relapse increases with the duration of follow-up.[8] We have shown a relapse rate of 28% after 5 years in a large group of 65 patients treated with cyclophosphamide for idiopathic membranous nephropathy, nephrotic syndrome and renal insufficiency.[8] Ponticelli found an even higher relapse rate of 30% after 2 years in patients with normal renal function treated with chlorambucil.[26] Therefore, especially regarding the efficacy in maintaining a remission of proteinuria, a larger group of patients and longer follow-up are needed. It will be relevant to determine if relapses occurring after MMF treatment respond to a second course of immunosuppression. We have shown before, that repeated treatment with cyclophosphamide is effective in patient who had received previous therapy with chlorambucil or cyclophosphamide.[27] In such case, it might always be worthwhile to use the less toxic drug as first line therapy.

Side effects were almost equally frequent but less severe with the use of MMF. Especially leucocytopenia was more frequent with CP-use and indeed the number of infections was less with the use of MMF. This is even more remarkable since half of the patients in the CP-treated group have used trimethoprim-sulfamethoxazole prophylactically. Cyclophosphamide is associated with bladder toxicity and malignancies[28;29], and these problems do not occur with MMF. As the risks of these serious side effects of cyclophosphamide increase with increasing cumulative dose, this could be an extra argument for starting with a less toxic drug like MMF, reserving CP for patients not responding on or relapsing after treatment with MMF.

In conclusion, the short-term efficacy of MMF and steroids for the treatment of patients with idiopathic membranous nephropathy, nephrotic syndrome and renal insufficiency seems comparable with the efficacy of cyclophosphamide and steroids. Our predefined treatment schedule with a longer duration of therapy (12 months), a higher dose of MMF (2 g/day) and the addition of steroids, seems to result in substantial reductions of proteinuria and improvement in renal function. Relapse rate is of serious concern. Results of larger groups of patients with longer follow-up and head to head comparisons between MMF and current immunosuppressive therapies for this group of patients have to be awaited.

Appendix

The following colleagues have actively participated in this study of the Membranous Nephropathy Study Group:

Dr. J. Broekroelofs, Medical Center Leeuwarden, Leeuwarden; Dr. P.G.G. Gerlag, Máxima Medical Center, Veldhoven and Dr. A.G. Lieverse, Máxima Medical Center, Eindhoven; Dr. W. Grave, Laurentius Hospital, Roermond; Dr. E.C. Hagen and Dr. C.A.J.M. Gaillard, Meander Medical Center, Amersfoort; Mrs. Dr. M.I. Koolen, Jeroen Bosch Hospital, ’s Hertogenbosch; Dr. L.J.M. Reichert, Rijnstate Hospital, Arnhem and Dr. A.L. Zanen, Deventer Hospital, Deventer.

References

1. Haas M, Meehan SM, Karrison TG, Spargo BH. Changing etiologies of unexplained adult nephrotic syndrome: a comparison of renal biopsy findings from 1976-1979 and 1995-1997. *Am J Kidney Dis* 1997; 30: 621-631
2. Donadio JV, Jr, Torres VE, Velosa JA, *et al*. Idiopathic membranous nephropathy: the natural history of untreated patients. *Kidney Int* 1988; 33: 708-715
3. Schieppati A, Mosconi L, Perna A, *et al*. Prognosis of untreated patients with idiopathic membranous nephropathy. *N Engl J Med* 1993; 329: 85-89
4. Ponticelli C, Zucchelli P, Passerini P, *et al*. A 10-year follow-up of a randomized study with methylprednisolone and chlorambucil in membranous nephropathy. *Kidney Int* 1995; 48: 1600-1604
5. Stirling CM, Simpson K, Boulton-Jones JM. Immunosuppression and outcome in idiopathic membranous nephropathy. *Q J Med* 1998; 91: 159-164
6. Branten AJ, Reichert LJ, Koene RA, Wetzels JF. Oral cyclophosphamide versus chlorambucil in the treatment of patients with membranous nephropathy and renal insufficiency. *Q J Med* 1998; 91: 359-366
7. Branten AJ, Wetzels JF. Short- and long-term efficacy of oral cyclophosphamide and steroids in patients with membranous nephropathy and renal insufficiency. Study Group. *Clin Nephrol* 2001; 56: 1-9
8. du Buf-Vereijken PW, Branten AJ, Wetzels JF. Cytotoxic therapy for membranous nephropathy and renal insufficiency: improved renal survival but high relapse rate. *Nephrol Dial Transplant* 2004; 19: 1142-1148
9. du Buf-Vereijken PW, Feith GW, Hollander D, *et al*. Restrictive use of immunosuppressive treatment in patients with idiopathic membranous nephropathy: high renal survival in a large patient cohort. *Q J Med* 2004; 97: 353-360
10. Jindal K, West M, Bear R, Goldstein M. Long-term benefits of therapy with cyclophosphamide and prednisone in patients with membranous glomerulonephritis and impaired renal function. *Am J Kidney Dis* 1992; 19: 61-67
11. Falk RJ, Hogan SL, Muller KE, Jennette JC. Treatment of progressive membranous glomerulopathy. A randomized trial comparing cyclophosphamide and corticosteroids with corticosteroids alone. The Glomerular Disease Collaborative Network. *Ann Intern Med* 1992; 116: 438-445
12. Torres A, Dominguez-Gil B, Carreno A, *et al*. Conservative versus immunosuppressive treatment of patients with idiopathic membranous nephropathy. *Kidney Int* 2002; 61: 219-227
13. Cattran DC. Idiopathic membranous glomerulonephritis. *Kidney Int* 2001; 59: 1983-1994
14. Pei Y, Cattran D, Greenwood C. Predicting chronic renal insufficiency in idiopathic membranous glomerulonephritis. *Kidney Int* 1992; 42: 960-966
15. Cattran DC, Pei Y, Greenwood CM, *et al*. Validation of a predictive model of idiopathic membranous nephropathy: its clinical and research implications. *Kidney Int* 1997; 51: 901-907
16. Reichert LJ, Koene RA, Wetzels JF. Prognostic factors in idiopathic membranous nephropathy. *Am J Kidney Dis* 1998; 31: 1-11
17. Branten AJ, du Buf-Vereijken PW, Klasen IS, *et al*. Urinary excretion of β_2-microglobulin and IgG predict prognosis in idiopathic membranous nephropathy: A validation study. *J Am Soc Nephrol* 2005; 16: 169-174

18. Reichert LJ, Koene RA, Wetzels JF. Urinary excretion of β_2-microglobulin predicts renal outcome in patients with idiopathic membranous nephropathy. *J Am Soc Nephrol* 1995; 6: 1666-1669

19. Reichert LJ, Koene RA, Wetzels JF. Urinary IgG excretion as a prognostic factor in idiopathic membranous nephropathy. *Clin Nephrol* 1997; 48: 79-84

20. Bazzi C, Petrini C, Rizza V, *et al*. Urinary excretion of IgG and alpha(1)-microglobulin predicts clinical course better than extent of proteinuria in membranous nephropathy. *Am J Kidney Dis* 2001; 38: 240-248

21. Briggs WA, Choi MJ, Scheel PJ, Jr. Successful mycophenolate mofetil treatment of glomerular disease. *Am J Kidney Dis* 1998; 31: 213-217

22. Briggs WA, Choi MJ, Scheel PJ, Jr. Follow-up on mycophenolate treatment of glomerular disease. *Am J Kidney Dis* 1998; 31: 898-899

23. Choi MJ, Eustace JA, Gimenez LF, *et al*. Mycophenolate mofetil treatment for primary glomerular diseases. *Kidney Int* 2002; 61: 1098-1114

24. Miller G, Zimmerman R, III, Radhakrishnan J, Appel G. Use of mycophenolate mofetil in resistant membranous nephropathy. *Am J Kidney Dis* 2000; 36: 250-256

25. Polenakovic M, Grcevska L, Dzikova S. Mycophenolate mofetil in treatment of idiopathic stages III-IV membranous nephropathy. *Nephrol Dial Transplant* 2003; 18: 1233-1234

26. Ponticelli C, Altieri P, Scolari F, *et al*. A randomized study comparing methylprednisolone plus chlorambucil versus methylprednisolone plus cyclophosphamide in idiopathic membranous nephropathy. *J Am Soc Nephrol* 1998; 9: 444-450

27. du Buf-Vereijken PW, Wetzels JF. Efficacy of a second course of immunosuppressive therapy in patients with membranous nephropathy and persistent or relapsing disease activity. *Nephrol Dial Transplant* 2004; 19: 2036-2043

28. Talar-Williams C, Hijazi YM, Walther MM, *et al*. Cyclophosphamide-induced cystitis and bladder cancer in patients with Wegener granulomatosis. *Ann Intern Med* 1996; 124: 477-484

29. Knight A, Askling J, Ekbom A. Cancer incidence in a population-based cohort of patients with Wegener's granulomatosis. *Int J Cancer* 2002; 100: 82-85

Chapter 5

Restrictive use of immunosuppressive treatment in patients with idiopathic membranous nephropathy: high renal survival in a large patient cohort

P.W.G. du Buf - Vereijken[1,2], G.W. Feith[3], D. Hollander[4],
P.G.G. Gerlag[5], J.J.J.M. Wirtz[6], T.C. Noordzij[7] and J.F.M. Wetzels[1]
for the Membranous Nephropathy Study Group

[1] Department of Nephrology, Radboud University Nijmegen Medical Center, Nijmegen,
[2] Department of Internal Medicine, Amphia Hospital, Breda,
[3] Department of Internal Medicine, Hospital Gelderse Vallei, Ede,
[4] Department of Internal Medicine, Jeroen Bosch Center, 's Hertogenbosch,
[5] Department of Internal Medicine, Máxima Medical Center, Veldhoven,
[6] Department of Internal Medicine, Laurentius Hospital, Roermond,
[7] Department of Internal Medicine, Franciscus Hospital, Roosendaal,
The Netherlands

Q J Med 2004; 97(6): 353-60

Summary

Background. Immunosuppressive treatment initiated at an early stage in patients with idiopathic membranous nephropathy (iMN) improves renal survival. Treatment should ideally be restricted to high-risk patients.

Aim. To evaluate the efficacy of a restrictive immunosuppressive treatment strategy for patients with iMN.

Design. Prospective cohort study evaluating a pre-defined treatment protocol.

Methods. From 1988, we adopted a restrictive treatment strategy: immunosuppressive treatment, mainly consisting of cyclophosphamide and steroids, was advised only in patients with renal insufficiency or severe intolerable nephrotic syndrome. We evaluated this strategy in a large patient cohort. To exclude any bias, we included all adult patients with iMN biopsied in the study period with a serum creatinine (Scr) < 135 μmol/l, a proteinuria ≥ 3.0 g/day and/or a serum albumin (Salb) ≤ 30 g/l at the time of biopsy. Analysis was according to the intention-to-treat principle.

Results. We studied 69 patients. At the time of biopsy, mean age was 51 years, Scr 90 μmol/l, Salb 23 g/l and proteinuria 6.7 g/day. Average follow-up was 5.5 years. Thus far 33 (48%) patients have received immunosuppressive therapy, mainly because of renal insufficiency (*n*=24). Status at the end of follow-up was: complete remission *n*=22 (32%), partial remission *n*=24 (35%), nephrotic syndrome *n*=15 (22%), persistent proteinuria *n*=1 (1.4%), ESRD *n*=6 (8.7%), death *n*=1 (1.4%; due to bladder carcinoma after cyclophosphamide therapy). Patient survival was 100% at 5 and 7 years. Renal survival was 94% at 5 years and 88% at 7 years.

Conclusion. In patients with iMN, a restrictive treatment policy assures a favourable prognosis, while preventing exposure to immunosuppressive therapy in > 50% of the patients.

Introduction

The treatment of patients with idiopathic membranous nephropathy is still a matter of debate. Some authors have argued against the use of immunosuppressive drugs.[1] A meta-analysis of randomized studies found no prove for a beneficial effect of immunosuppressive therapy on renal survival.[2] However, the recent publication of the long-term follow-up data of the randomized, controlled trial conducted by Ponticelli and his collaborators has provided hard evidence that immunosuppressive therapy is effective and improves renal survival.[3,4] In the latter study, treatment, which consisted of a combination of chlorambucil and steroids, was started at an early stage, i.e. before significant deterioration of renal function had occurred. Since only up to 40-50% of untreated patients will progress to end-stage renal disease (ESRD), such a strategy of early treatment start will expose many patients unnecessarily to immunosuppressive treatment.[1,4-10] In view of the potential side effects of therapy, many investigators have been reluctant to adopt the Ponticelli treatment strategy, although the efficacy of their regimen is not debated. Uncontrolled studies have suggested that immunosuppressive therapy is effective even when started in patients with established renal insufficiency.[11-21] We and others have shown that immunosuppressive treatment offers a clear renal survival benefit over untreated historic control patients.[21,22] However, thus far there are no data to suggest that such a restrictive treatment policy is safe and assures an outcome which is comparable to that obtained by Ponticelli *et al*.

The present report summarizes the efficacy of such a restrictive treatment strategy applied to a large cohort of patients with idiopathic membranous nephropathy, a nephrotic syndrome and normal renal function at the time of renal biopsy.

Methods

Treatment Strategy

For more than two decades, we have been actively recruiting patients with idiopathic membranous nephropathy for participation in ongoing studies directed at the identification of prognostic risk factors.[23,24] Treatment guidelines have been developed for the follow-up of these patients. Until 1988, most patients were treated with alternate-day high-dose prednisone

monotherapy.[25] From 1988 onwards, we used a more restrictive treatment strategy. Immunosuppressive therapy was advised only in patients with renal insufficiency (serum creatinine > 135 μmol/l and/or an increase in serum creatinine of > 50%) or severe intolerable nephrotic syndrome (prolonged proteinuria ≥ 8 g/day). Details of our immunosuppressive treatment regimens have been described.[11-13,26] Initially, immunosuppressive therapy consisted of the combination of chlorambucil and corticosteroids, or a combination of i.v. cyclophosphamide and i.v. methylprednisolone. The latter combination was ineffective.[26] In 1991, we started to use a combination of oral cyclophosphamide and steroids, and this treatment has been the treatment of choice after an analysis of our data suggested that cyclophosphamide was more effective and better tolerated than chlorambucil.[12] Cyclophosphamide treatment consisted of oral cyclophosphamide in a dose of 1.5-2 mg/kg bodyweight/day for 12 months. In most patients, the corticosteroid regimen consisted of three consecutive i.v. pulses of 1 g of methylprednisolone at the beginning of the first, third and fifth month of therapy, and oral prednisone in a dose of 0.5 mg/kg bodyweight on alternate days for six months. Repeated courses of immunosuppressive therapy were offered to patients in whom previous therapy showed no effect, or who relapsed to nephrotic range proteinuria, together with a rise in serum creatinine of more than 50% over the lowest value attained during or after the previous course of immunosuppressive treatment. The study protocol, on the treatment of patients with membranous nephropathy, has been approved by the University Hospital Ethics Committee.

Patient Selection

To exclude any bias, we have identified all patients with membranous nephropathy from the pathology registries of our university hospital and five referring hospitals. We have included only patients with a first renal biopsy in the study period, age ≥ 18 years, and with a serum creatinine < 135 μmol/l, a proteinuria ≥ 3.0 g/10 mmol creatinine and/or a serum albumin ≤ 30 g/l at the time of biopsy. Follow-up should have lasted at least 6 months. We excluded patients with a secondary membranous nephropathy on clinical and laboratory grounds. Patients were followed prospectively at their local hospitals at regular intervals. For this study, we have evaluated the patient records, retrieved relevant laboratory data and when applicable, we have specified the time of start, the type and the duration of immunosuppressive therapy. The indication for starting immunosuppression was noted.

Follow-up started at the time of renal biopsy and continued until December 2002, or ended at the time of death or onset of ESRD.

Calculations and Statistics

For descriptive statistics, results are given as means ± SD or medians with range when appropriate. Creatinine clearance was calculated according to Cockcroft and Gault.[27] Proteinuria was expressed per 10 mmol creatinine (protein-creatinine index). Complete remission of proteinuria (CR), partial remission (PR), persistent proteinuria (PP) and nephrotic range proteinuria (NS) were defined as protein-creatinine indices of ≤ 0.2, 0.21-2.0, 2.1-3.4 and ≥ 3.5 g/10 mmol creatinine, respectively, where in case of remission, renal function should have improved or at least stabilized. Mean arterial pressure (MAP) was calculated as diastolic blood pressure plus one third of the pulse pressure (systolic minus diastolic blood pressure). For calculations of renal survival, the time of renal death was defined as the start of renal replacement therapy. The cumulative probabilities of death and ESRD were estimated according to Kaplan and Meier.

Results

In the period from 1988 to 2002, idiopathic membranous nephropathy was diagnosed in 87 patients. For this study, we excluded 18 patients because of renal insufficiency (n=8; median serum creatinine at time of biopsy 218 [149-324] µmol/l) or non-nephrotic proteinuria (n=5) at the time of renal biopsy, age < 18 years (n=2) or follow-up < 6 months (n=3). Thus the study cohort comprised 69 patients.

Patient characteristics at the time of renal biopsy are given in Table 1.

Mean follow-up after renal biopsy was 5.4 (range 0.5-14.1) years, 37 patients have been followed for > 5 years, nine for > 10 years. Four patients were lost to follow-up: two because they moved (one in CR, one with persistent proteinuria), one because of non-compliance (in PR) and one for unknown reason (with persistent nephrotic syndrome).

Table 1. Characteristics of the patients at the time of biopsy

Characteristic	Value
Number of patients	69
Male : female	43 : 26
Age (years)	51 ± 15
Serum creatinine (μmol/l)	90 ± 20
Serum albumin (g/l)	23 ± 5
Serum cholesterol (mmol/l)	10.4 ± 3.1
Proteinuria (g/10 mmol creatinine)	6.7 ± 3.0
Creatinine Clearance (ml/min/1.73 m^2)	99 ± 33
Mean Arterial Pressure (mm Hg)	103 ± 13

Data are means ± SD where appropriate.

Follow-up, with emphasis on the use of immunosuppressive therapy, is detailed in Figure 1. Thus far, 33 patients have been treated with immunosuppressive drugs. In 24 patients, immunosuppressive therapy was started because of renal insufficiency. For these patients, the mean time between renal biopsy and start of the immunosuppressive therapy amounted to 11 (range 0.5-103) months and total follow-up time from renal biopsy was 65 (range 16-169) months. If we analyse the data according the intention-to-treat principle, patient survival was 100% at 5 and 7 years, and renal survival 94% at 5 years and 88% at 7 years (Figures 2 and 3). At the end of follow-up, 22 patients were in complete remission (32%), 24 in partial remission (35%), one patient had persistent proteinuria (1.4%) and 15 patients had a nephrotic syndrome (22%). Six patients had progressed to ESRD (8.7%) and one patient had died (1.4%), due to disseminated bladder carcinoma, occurring after a cumulative dose of only 20 g cyclophosphamide.

It is evident from Figure 1 that the advised treatment protocol has not been adhered to by 13 patients (white boxes in Figure 1). In three patients with established renal insufficiency, no immunosuppressive therapy was given because of old age (n=2; 73 and 83 years old, respectively) or patient refusal (n=1). Two patients with proteinuria < 8 g/day were treated with prednisone.

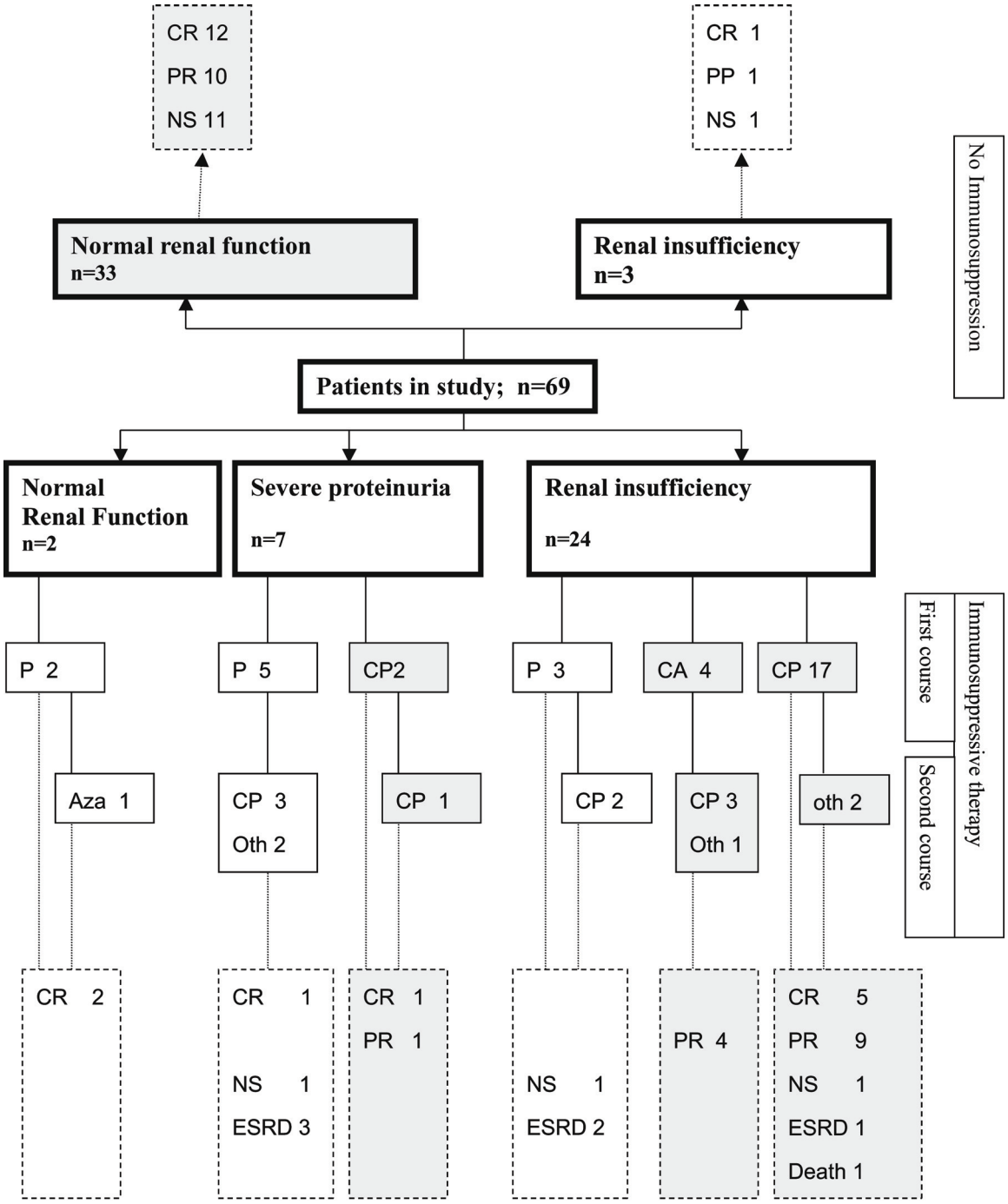


Figure 1. Flow-chart of treatment regimen.

Immunosuppressive therapies: P, prednisone; CP, cyclophosphamide with steroids;
CA, chlorambucil with steroids; Aza, azathioprine; oth, others.
CR, complete remission; PR, partial remission; NS, nephrotic syndrome; PP, persistent proteinuria;
ESRD, end-stage renal disease.
Dotted rectangles represent status at end of follow-up.
Gray rectangles indicate patients in whom the optimal treatment regimen was followed.

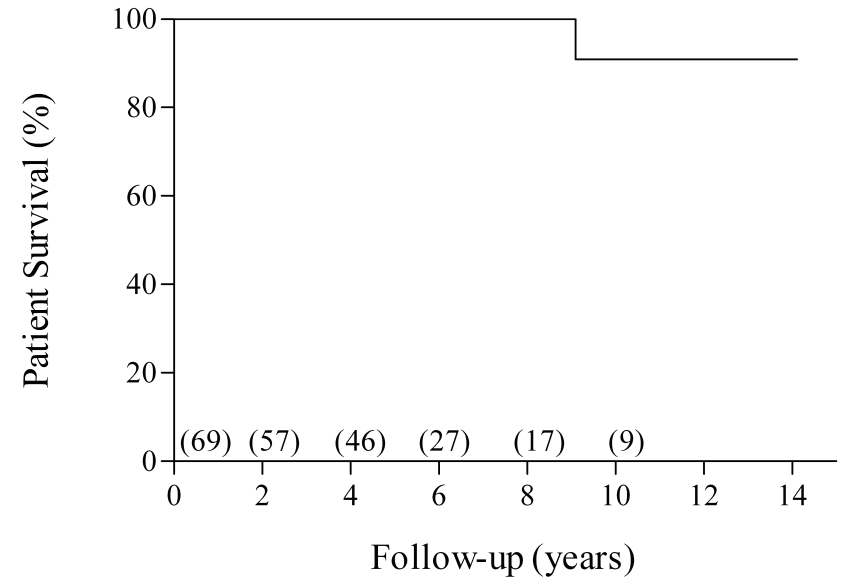


Figure 2. Patient survival.

Numbers of patients at risk are given in parentheses.

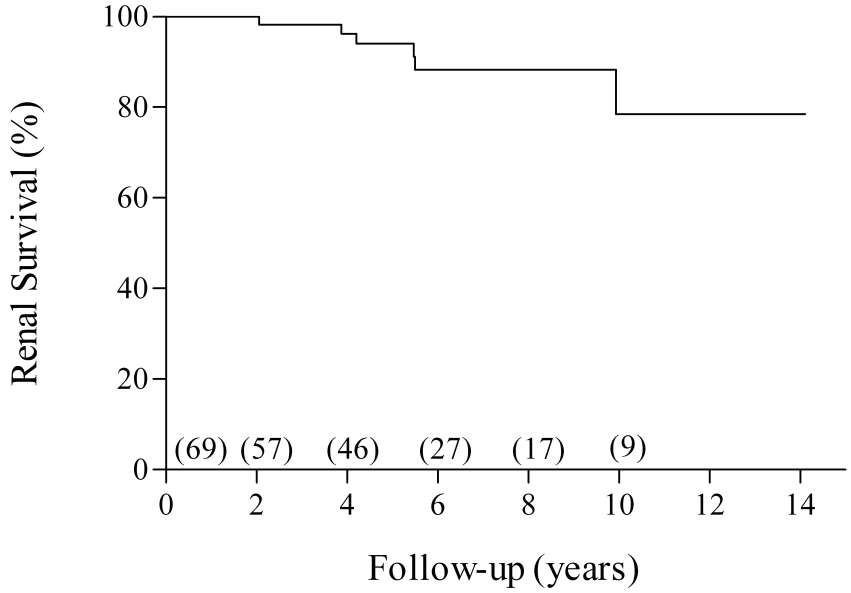


Figure 3. Renal survival.

Numbers of patients at risk are given in parentheses.

Furthermore, eight patients with severe nephrotic syndrome (*n*=5) or renal insufficiency (*n*=3) were treated initially with prednisone monotherapy, reflecting the reluctance of patients and/or doctors to use alkylating agents, especially in patients with preserved renal function. As expected, prednisone monotherapy proved ineffective in seven out of eight patients, thus necessitating a second, more aggressive course of immunosuppressive therapy. In four of these patients, serum creatinine exceeded 200 μmol/l at the time that the second course of therapy was started. To determine the potential influence of these protocol violations on patient outcome, we have analysed our data for the subgroup of patients who were treated according to the predefined protocol (*n*=56; 82%) thus including untreated patients with preserved renal function (*n*=33) and patients treated with a combination of steroids and cyclophosphamide or chlorambucil because of renal insufficiency (*n*=21) or a severe nephrotic syndrome (*n*=2). These patients are indicated in Figure 1 with gray boxes. In this subgroup analysis, patient survival was 100% at 5 and 7 years and 89% at 10 years, whereas renal survival was 97% at 5 and 7 years and 86% at 10 years follow-up.

Seven patients were initially treated with prednisone (*n*=5) or cyclophosphamide (*n*=2) because of severe proteinuria with normal renal function. Five of them progressed to renal insufficiency necessitating a second course of immunosuppression; four had a persistent proteinuria > 5.3 g/10 mmol creatinine after the start of the first course. In these five progressors, serum creatinine at the start of the first and at the start of the second immunosuppressive course was 85 (range 79-115) and 189 (range 162-489) μmol/l ($P < 0.01$) and proteinuria 9.1 (range 8.1-46.0) and 6.6 (range 5.3-13.0) g/10 mmol creatinine (P = NS), respectively.

Discussion

Our data clearly show that a restrictive treatment strategy applied to nephrotic patients with idiopathic membranous nephropathy results in high patient and renal survival. We feel that our study thus provides some arguments against the unrestrictive use of immunosuppressive therapy in patients with idiopathic membranous nephropathy.

Admittedly, our study is not a randomized, controlled trial, but a cohort study. We feel that the data are representative. Our patient cohort is large, and included only patients who were nephrotic at the time of biopsy. To exclude any referral bias, we have retrieved all patients

who were diagnosed with idiopathic membranous nephropathy in the study period, using the pathology registries. Furthermore, in our analysis we have included the data of all patients, irrespective of their course and given treatment (intention-to-treat principle). Our data must be compared with those reported by Ponticelli *et al*.[3,4] The Italian investigators have conducted a randomized, controlled study in patients with idiopathic membranous nephropathy and nephrotic syndrome, who were randomized for either no treatment or treatment with a combination of chlorambucil and steroids. This study provided hard evidence that unrestricted immunosuppressive treatment improves renal survival. The baseline characteristics of the patients in Ponticelli's and our study were quite similar with respect to proteinuria and renal function (Table 2).

Table 2. Results of different treatment strategies in patients with idiopathic membranous nephropathy and nephrotic syndrome

	This Study	Ponticelli untreated[3,4]	Ponticelli treated[3,4]	Stirling[19]
n	69	39	42	53
Treated with immunosuppressives	48%	0%	100%	36%
Immunosuppressive drug	Mainly cyclophosphamide	-	Chlorambucil	Chlorambucil
Serum creatinine (µmol/l)				
At biopsy	90 ± 20	93 ± 25	94 ± 22	130
At start immunosuppressive therapy	150 ± 54		94 ± 22	267
Proteinuria at biopsy (g/10 mmol creatinine)	6.7 ± 3.0	5.3 ± 2.8	6.2 ± 3.0	8.3
Follow-up (years)	5.4 [0.5-14.1]	7.3*	> 10*	5.9
Remissions: CR	22 (32%)	2 (5.1%)	17 (40%)	13 (24%)
PR	24 (35%)	11 (28%)	9 (21%)	12 (23%)
NS / Renal dysfunction	16 (23%)	14 (36%)	13 (31%)	13 (25%)
ESRD	6 (8.7%)	9 (23%)	2 (4.8%)	7 (13%)
Death (non-renal)	1 (1.4%)	3 (8%)	1 (2.4%)	8 (15%)
Renal survival				
5 years	94%	84%*	97%*	84%
7 years	88%	79%*	94%*	72%*
10 years	78%	60%	92%	54%

CR, complete remission; PR, partial remission; NS, nephrotic syndrome; ESRD, end-stage renal disease. *Approximate values derived from graphs. Values are means ± SD, or medians [range].

It is evident from Table 2 that the outcome in our patient cohort was better than in the untreated group of patients from Ponticelli's study, thus supporting the efficacy of immunosuppressive therapy. Most importantly, however, our patient cohort fared almost as well as the treated patients with respect to remission rate and development of renal failure. Admittedly, follow-up in our study was less than the 10-year follow-up reported by Ponticelli *et al*. The estimated 10-year renal survival in our patients is 78% (95% CI 58-98%), clearly lower than the 92% survival rate reported by Ponticelli *et al*. (95% CI not provided). These differences in renal survival can be attributed to the fact that, especially in the initial study period, some of our patients received the less effective prednisone monotherapy. If we analysed our data 'per protocol', renal survival was 97% at 7 years and 86% at 10 years. In our treated patients, serum creatinine at the start of immunosuppressive therapy averaged 150 ± 54 μmol/l.

The outcome in our patient cohort is clearly better than that reported by Stirling *et al*.[19] These authors reported the outcome in a group of patients with idiopathic membranous nephropathy, in whom immunosuppressive treatment, which consisted of a combination of chlorambucil and steroids, was restricted to patients with renal insufficiency. Although the number of remissions was higher and the percentage of patients reaching ESRD lower than in a historical control group of untreated patients, the differences did not reach statistical significance. We feel that these seemingly discordant results can be explained (Table 2). Stirling *et al.* initiated immunosuppressive therapy at a late stage, serum creatinine averaging 267 μmol/l at start of treatment. Furthermore, they did not use intravenous methylprednisolone in all their patients, and in earlier publications they have indicated that the outcome in patients not receiving i.v. methylprednisolone is not as good.[28] Last but not least, the type of immunosuppression is important. We have previously demonstrated that in patients with renal insufficiency chlorambucil is less well tolerated and less effective than cyclophosphamide.[12]

Our data thus indicate that it is unnecessary to use immunosuppressive treatment in all patients with membranous nephropathy. On the other hand, it is evident from the data presented in Table 2, that the treatment should be started before severe renal insufficiency has developed. We feel that the good renal survival in our patients who were treated per protocol suggests that treatment should be started as soon as deterioration of renal function becomes apparent, as reflected by a rise of serum creatinine > 50% or a value of serum creatinine > 135 μmol/l. It might well be that starting therapy at an earlier time point might even prove

slightly more effective. In this respect it could be advantageous to identify patients at high risk for renal insufficiency at an earlier stage, using prognostic markers such as urinary excretion of IgG, β_2-microglobulin or α_1-microglobulin.[23,24,29]

When comparing our treatment schedule, consisting of 12 months of cyclophosphamide with the Ponticelli regimen (3 months of chlorambucil or cyclophosphamide), safety issues are a concern. If cyclophosphamide is given for $>$ 3 months, there is an increasing risk of persistent amenorrhea and azoospermia. Therefore, in patients who wish to become pregnancy, we currently replace cyclophosphamide by azathioprine after 3 months. Bladder cancer is also a serious complication of long-term cyclophosphamide therapy, although bladder cancer is particularly observed if duration of treatment exceeds 2 years and the cumulative dosage exceeds 100 g.[30,31] Our current regimen contains approximately 40 g of cyclophosphamide. Although it would be tempting to use the 3 months regimen, we are somewhat concerned about the efficacy. In fact, we have demonstrated that 12 months of cyclophosphamide is more effective (and less toxic) than 3 months of chlorambucil.[12] Thus, it is quite possible that the efficacy of a drug regimen is dependent on the duration of treatment. Furthermore, the efficacy of the Ponticelli regimen has only been demonstrated in low-risk patients.[3,4,32] The optimal timing and dosage of cyclophosphamide therapy is an important topic for future studies. Until these issues are resolved, we favour a 12-month regimen as initial treatment in patients with membranous nephropathy and renal insufficiency.

Conclusions

In patients with idiopathic membranous nephropathy and nephrotic syndrome, a restrictive use of immunosuppressive therapy assures a favourable prognosis while preventing exposure to immunosuppression in over half of the patients. The optimal time of start of immunosuppressive therapy needs to be further defined.

Acknowledgements

The following colleagues actively participated in this study:
Dr J.J. Beutler, Dr J.L.J. Jansen and Mrs Dr M.I. Koolen, Jeroen Bosch Hospital, 's Hertogenbosch; Dr M. den Hartog, Hospital Gelderse Vallei, Ede; Dr A.W.L. van den Wall Bake, Máxima Medical Center, Veldhoven; Dr W. Grave, Laurentius Hospital, Roermond; Dr H.P.C. van Roermund, Franciscus Hospital, Roosendaal.

References

1. Schieppati A, Mosconi L, Perna A, *et al.* Prognosis of untreated patients with idiopathic membranous nephropathy. *N Engl J Med* 1993; 329: 85-89
2. Imperiale TF, Goldfarb S, Berns JS. Are cytotoxic agents beneficial in idiopathic membranous nephropathy? A meta-analysis of the controlled trials. *J Am Soc Nephrol* 1995; 5: 553-558
3. Ponticelli C, Zucchelli P, Passerini P, *et al.* A randomized trial of methylprednisolone and chlorambucil in idiopathic membranous nephropathy. *N Engl J Med* 1989; 320: 8-13
4. Ponticelli C, Zucchelli P, Passerini P, *et al.* A 10-year follow-up of a randomized study with methylprednisolone and chlorambucil in membranous nephropathy. *Kidney Int* 1995; 48: 1600-1604
5. Davison AM, Cameron JS, Kerr DN, *et al.* The natural history of renal function in untreated idiopathic membranous glomerulonephritis in adults. *Clin Nephrol* 1984; 22: 61-67
6. MacTier R, Boulton Jones JM, Payton CD, McLay A. The natural history of membranous nephropathy in the West of Scotland. *Q J Med* 1986; 60: 793-802
7. Donadio JV, Jr, Torres VE, Velosa JA, *et al.* Idiopathic membranous nephropathy: the natural history of untreated patients. *Kidney Int* 1988; 33: 708-715
8. Cattran DC, Delmore T, Roscoe J, *et al.* A randomized controlled trial of prednisone in patients with idiopathic membranous nephropathy. *N Engl J Med* 1989; 320: 210-215
9. Cameron JS, Healy MJ, Adu D. The Medical Research Council trial of short-term high-dose alternate day prednisolone in idiopathic membranous nephropathy with nephrotic syndrome in adults. The MRC Glomerulonephritis Working Party. *Q J Med* 1990; 74: 133-156
10. Honkanen E, Tornroth T, Gronhagen-Riska C, Sankila R. Long-term survival in idiopathic membranous glomerulonephritis: can the course be clinically predicted? *Clin Nephrol* 1994; 41:127-134
11. Wetzels JF, Reichert LJ. Efficacy of immunosuppressive treatment in patients with membranous nephropathy and renal insufficiency. *Kidney Int Suppl* 1997; 61: S63-S6
12. Branten AJ, Reichert LJ, Koene RA, Wetzels JF. Oral cyclophosphamide versus chlorambucil in the treatment of patients with membranous nephropathy and renal insufficiency. *Q J Med* 1998; 91: 359-366
13. Branten AJ, Wetzels JF. Short- and long-term efficacy of oral cyclophosphamide and steroids in patients with membranous nephropathy and renal insufficiency. *Clin Nephrol* 2001; 56: 1-9
14. Bruns FJ, Adler S, Fraley DS, Segel DP. Sustained remission of membranous glomerulonephritis after cyclophosphamide and prednisone. *Ann Intern Med* 1991; 114: 725-730
15. Jindal K, West M, Bear R, Goldstein M. Long-term benefits of therapy with cyclophosphamide and prednisone in patients with membranous glomerulonephritis and impaired renal function. *Am J Kidney Dis* 1992; 19: 61-67
16. Mathieson PW, Turner AN, Maidment CG, *et al.* Prednisolone and chlorambucil treatment in idiopathic membranous nephropathy with deteriorating renal function. *Lancet* 1988; 2: 869-872
17. Brunkhorst R, Wrenger E, Koch KM. Low-dose prednisolone/chlorambucil therapy in patients with severe membranous glomerulonephritis. *Clin Investig* 1994; 72: 277-282

18. Bone JM, Rustom R, Williams PS. 'Progressive' versus 'indolent' idiopathic membranous glomerulonephritis. *Q J Med* 1997; 90: 699-706

19. Stirling CM, Simpson K, Boulton-Jones JM. Immunosuppression and outcome in idiopathic membranous nephropathy. *Q J Med* 1998; 91: 159-164

20. Cattran DC. Idiopathic membranous glomerulonephritis. *Kidney Int* 2001; 59**:** 1983-1994

21. Du Buf-Vereijken PWG, Branten AJW, Wetzels JFM. Cytotoxic therapy for membranous nephropathy and renal insufficiency: improved renal survival but high relapse rate. *Nephrol Dial Tansplant* 2004; 19: 1142-1148

22. Torres A, Dominguez-Gil B, Carreno A, *et al.* Conservative versus immunosuppressive treatment of patients with idiopathic membranous nephropathy. *Kidney Int* 2002; 61: 219-227

23. Reichert LJ, Koene RA, Wetzels JF. Urinary excretion of β_2-microglobulin predicts renal outcome in patients with idiopathic membranous nephropathy. *J Am Soc Nephrol* 1995; 6: 1666-1669

24. Reichert LJ, Koene RA, Wetzels JF. Urinary IgG excretion as a prognostic factor in idiopathic membranous nephropathy. *Clin Nephrol* 1997; 48: 79-84

25. A controlled study of short-term prednisone treatment in adults with membranous nephropathy. Collaborative Study of the Adult Idiopathic Nephrotic Syndrome. *N Engl J Med* 1979; 301: 1301-1306

26. Reichert LJ, Huysmans FT, Assmann K, *et al.* Preserving renal function in patients with membranous nephropathy: daily oral chlorambucil compared with intermittent monthly pulses of cyclophosphamide. *Ann Intern Med* 1994; 121: 328-333

27. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. *Nephron* 1976; 16: 31-41

28. Warwick GL, Geddes CG, Boulton-Jones JM. Prednisolone and chlorambucil therapy for idiopathic membranous nephropathy with progressive renal failure. *Q J Med* 1994; 87: 223-229

29. Bazzi C, Petrini C, Rizza V, *et al.* Urinary excretion of IgG and alpha-1-microglobulin predicts clinical course better than extent of proteinuria in membranous nephropathy. *Am J Kidney Dis* 2001; 38: 240-248

30. Talar-Williams C, Hijazi YM, Walther MM, *et al.* Cyclophosphamide-induced cystitis and bladder cancer in patients with Wegener granulomatosis. *Ann Intern Med* 1996; 124: 477-484

31. Knight A, Askling J, Ekbom A. Cancer incidence in a population-based cohort of patients with Wegener's granulomatosis. *Int J Cancer* 2002; 100: 82-85

32. Ponticelli C, Altieri P, Scolari F, *et al*. A randomized study comparing methylprednisolone plus chlorambucil versus methylprednisolone plus cyclophosphamide in idiopathic membranous nephropathy. *J Am Soc Nephrol* 1998; 9: 444-450

Chapter 6

Urinary excretion of ß$_2$-microglobulin and IgG predict prognosis in idiopathic membranous nephropathy: a validation study

Amanda J.W. Branten[1], Peggy W.G. du Buf-Vereijken[1,3], Ina S. Klasen[2], Frank H. Bosch[4], Geert W. Feith[5], Daan A. Hollander[6] and Jack F.M. Wetzels[1]

[1]Department of Nephrology and [2]Department of Clinical Chemistry, Radboud University Nijmegen Medical Center, Nijmegen,
[3] Department of Internal Medicine, Amphia Hospital, Breda,
[4]Department of Internal Medicine, Hospital Rijnstate, Arnhem,
[5]Department of Internal Medicine, Hospital Gelderse Vallei, Ede,
[6]Department of Internal Medicine, Jeroen Bosch Hospital, 's-Hertogenbosch,
The Netherlands

J Am Soc Nephrol 2005; 16(1): 169-174

Abstract

An accurate prediction of the prognosis of patients with idiopathic membranous nephropathy (iMN) should allow restriction of immunosuppressive treatment to patients who are at highest risk for ESRD. On the basis of retrospective studies, it has previously been suggested that the urinary excretions of β_2-microglobulin ($U\beta_2m$) and IgG (UIgG) are useful predictors of renal insufficiency in patients with iMN. The threshold values of 0.5 μg/min ($U\beta_2m$) and 250 mg/24 h (UIgG) have been validated in a new and larger patient cohort. From 1995 onward, 57 patients with iMN (38 men, 19 women; age 48 ± 16 yr), a nephrotic syndrome, and a serum creatinine level ≤ 1.5 mg/dl were studied prospectively. At baseline, a standardized measurement was carried out to determine renal function and protein excretion. The end point renal death was defined as a serum creatinine exceeding 1.5 mg/dl or a rise of serum creatinine of > 50%. Mean (±SD) follow-up was 53 ± 23 months. Thus far, 25 (44%) of the patients have reached the end point renal death. Multivariate analysis confirmed $U\beta_2m$ as the strongest independent predictor for the development of renal insufficiency. Sensitivity and specificity were 88% and 91%, respectively, for $U\beta_2m$, and both were 88% for UIgG. When the excretions of both proteins were combined, specificity improved to 97%. It is concluded that the present data validate the accuracy of $U\beta_2m$ and of UIgG in predicting renal outcome in patients with iMN. These markers can be used to guide decisions on the start of immunosuppressive treatment.

Introduction

Idiopathic membranous nephropathy (iMN) is one of the most frequent causes of the nephrotic syndrome in adults.[1] If left untreated, up to 40% of patients will progress to ESRD.[2-4] The efficacy of immunosuppressive therapy has been demonstrated in a randomized, controlled trial.[4] Although this study provided arguments to treat all patients with iMN and a nephrotic syndrome, most authors advocate restricting immunosuppressive treatment to patients who are at highest risk for developing ESRD.[5,6] It is well established that deterioration of renal function is a powerful predictor of ESRD.[7,8] Therefore, a trial of immunosuppressive therapy is warranted in patients with iMN and established renal insufficiency. However, it is evident that immunosuppressive treatment started at a relatively late time point may be less effective in attaining normal renal function.[9] Moreover, we and others have noted that the use of immunosuppressive agents in patients with renal insufficiency was associated with more frequent and more severe side effects than in patients who are treated in an earlier phase of their disease.[10-12] Therefore, it would be ideal if treatment could be optimized by identifying high-risk patients at an earlier time point. In patients with iMN, various risk factors for the development of renal failure have been identified.[13] However, the sensitivity and specificity of most of these factors (*e.g.*, age, gender, glomerular injury, tubular interstitial fibrosis) are too low to justify their use to guide decisions on the start of immunosuppressive therapy. Thus far, the level and the duration of proteinuria are the best predictive factor in a model introduced by the Toronto Glomerulonephritic Registry.[14] This model requires a minimal observation period of 6 to 18 months.

On the basis of data derived from small patient cohorts, we demonstrated previously that the urinary excretion of β_2-microglobulin (Uβ_2m) and IgG (UIgG), assessed in a single urine sample, independently predicted the development of renal insufficiency in patients with iMN.[15,16] Our data suggested high sensitivities and specificities, which ranged from 80 to 90%. We now have validated these results in a prospectively studied, new and larger patient cohort.

Materials and Methods

In our center, patients with proteinuria are evaluated using a standard protocol. In all of these patients, standardized urine and blood measurements are carried out as described below. For the validation study, we prospectively studied patients with biopsy-proven iMN, evaluated from 1995 onward. In the analysis, we included only patients with a baseline serum creatinine ≤ 1.5 mg/dl* and proteinuria ≥ 2.7 g/g creatinine and/or serum albumin ≤ 3.0 g/dl. We excluded patients who had been treated with immunosuppressive drugs other than oral prednisone. Patients were also excluded when the interval between renal biopsy and the baseline measurement exceeded 3 years.

Standardized Measurement of Urinary Proteins

Patients come to the ward after an overnight fast. Patients are instructed to take 4000 mg of sodium bicarbonate on the evening before to ensure that urinary pH exceeds 6.0, which is mandatory for the measurement of Uβ_2m. On the morning of the measurement, patients are not allowed to take diuretics. Upon arrival, 375 to 500 ml of tap water is given to enforce diuresis. The patients remain supine during 2 h except for voiding. Blood pressure measurements are done using an automatic device, and 10 consecutive readings are registered with an interval of 5 minutes (DINAMAP, Criticon, Tampa FL). Timed urine samples are collected, and in the middle of the collection period, a blood sample is drawn. In addition, two 24-h urine samples are collected for assessment of daily excretion of total protein and creatinine.

Laboratory Measurements

In the blood samples, we assessed the following parameters: creatinine, cholesterol, β_2m, albumin, IgG, and transferrin. In the timed urine samples, we measured creatinine, β_2m, α_1-microglobulin, albumin, IgG, and transferrin. The concentrations of serum creatinine, serum cholesterol, urinary total protein, and urinary creatinine were measured with standard automated techniques.

* Note: To convert serum creatinine in mg/dl to μmol/l, multiply by 88.4.

The concentrations of albumin, transferrin, α_1-microglobulin, 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). Urinary and serum β_2m were measured by ELISA as described before.[17]

Calculations

Endogenous creatinine clearance (ECC) was calculated according to the formula Ucr x V / Pcr, where Ucr is the concentration of creatinine in the urine, V is the urine flow, and Pcr is the plasma concentration of creatinine, and was corrected for body surface area. Because 24-h urine samples were not collected regularly during follow-up, we estimated creatinine clearances using the Cockcroft and Gault formula. In addition, we calculated GFR for patients who reached the end point renal death by applying the recently developed Modification of Diet in Renal Disease (MDRD) formula using serum creatinine, age, gender, race, serum albumin and serum urea.[18] The mean arterial pressure was the average of the last six of 10 registered measurements.

The amounts of β_2m, α_1-microglobulin, IgG, transferrin, and albumin in the timed urine samples are expressed as excretion per unit time (minute or 24 h). Protein selectivity index was calculated as the clearance of IgG divided by the clearance of transferrin. The total protein excretion in the 24-h urine samples was expressed as g/g creatinine to correct for sampling errors.

Statistical Analysis

For the validation study, we calculated renal survival using Kaplan-Meier statistics. Renal death was defined as an increase of serum creatinine > 50 % or an increase of serum creatinine > 1.5 mg/dl. Survival was calculated using the date of the baseline study at $t = 0$. We compared renal survival using log-rank test for patients with low and high Uβ_2m and UIgG. We used the threshold values established in our previous studies.[15,16] The threshold level for β_2m excretion was 0.5 µg/min and for IgG was 250 mg/24 h. Using these threshold levels, we calculated sensitivity, specificity, true positive predictive value, and true negative predictive value.

Because the use of a fixed serum creatinine value as end point, irrespective of the baseline value, might have introduced a bias (a subtle increase in serum creatinine could have been defined as failure), we performed a subanalysis in a group of patients with a baseline serum creatinine < 1.2 mg/dl.

Using the data of the present patient cohort, we also studied the effect of other parameters in predicting renal outcome. Univariate analysis and multivariate analysis using the Cox proportional hazard model with a forward stepwise procedure was performed to identify independent predictive parameters. Receiver operating characteristics (ROC) curves were made to determine the area under the curve (AUC) and to calculate the sensitivity and the specificity using the most discriminative thresholds. The following parameters were plotted into ROC curves: β_2m excretion, IgG excretion, α_1-microglobulin excretion, transferrin excretion, albumin excretion, selectivity index, ECC, serum creatinine, serum albumin, and total proteinuria per 24 h. The parameters with the highest AUC were selected and used as covariates in the Cox regression analysis. All values are given as means (±SD) or medians (range) when appropriate. All statistics were performed using SPSS software (SPSS version 11.0, Chicago, IL, USA). $P < 0.05$ was considered significant.

Results

From 1995 to 2002, we studied 57 patients who had iMN and fulfilled the inclusion criteria. In 90% of the patients, the baseline measurement was performed within 1 year after renal biopsy. Baseline characteristics are given in Table 1.

Two patients had been treated with prednisone. Patients have been followed for 53 ± 23 months. Thus far, 25 (44 %) patients have reached the predefined end point of renal death. The reason for renal death was a serum creatinine > 1.5 mg/dl in 21 patients and a rise of > 50% of serum creatinine in 4 patients. Overall renal survival was 81% at 6 months, 68% at 1 year, and 54% at 3 years. Thus, in most patients, progressive disease was apparent within 3 years after the baseline study. In this new patient cohort, the use of the previously established threshold values of Uβ_2m and UIgG excretion allowed an accurate prediction of renal outcome. Renal survival curves are depicted in Figures 1 and 2.

Table 1. Baseline characteristics of patients with iMN (n=57)

Gender	(M / F)	38 / 19
Age	(years)	48 ± 16
MAP	(mmHg)	98 ± 16
ECC - 24 h	(ml/min per 1.73 m^2)	88 ± 26
Serum creatinine	(mg/dl)	1.00 ± 0.23
Serum β_2m	(mg/l)	2.8 ± 1.1
Serum albumin	(g/dl)	2.4 ± 0.5
Cholesterol	(mg/dl)	329 ± 76
Interval Bx–measurement	(months)	2 (0–33)
Follow-up[a]	(months)	53 ± 23
Timed urine sample:		
albumin excretion	(mg/min)	3.8 (0.3–16)
IgG excretion	(mg/24 h)	197 (18–3597)
β_2m excretion	(µg/min)[b]	0.38 (0.05–68.4)
α_1m excretion	(µg/min)	29 (4–418)
transferrin excretion	(µg/min)	283 (17–1455)
Selectivity Index		0.18 (0.06–0.39)
proteinuria	(g/g creatinine)	5.8 (1.7–13.3)

Data are means ± SD or medians (range).
iMN, idiopathic membranous nephropathy; MAP, mean arterial pressure; ECC - 24 h, creatinine clearance calculated from 24 h urine; β_2m, β_2-microglobulin; α_1m, α_1-microglobuline; Bx, renal biopsy.
[a]From baseline measurement until end of follow-up.
[b]In case of β_2m excretion: n=56; in one patient, β_2m was not measurable because pH urine was too low (< 6.0).

Our calculations confirmed the high sensitivity and specificity (Table 2).

We evaluated the possible bias of using the fixed serum creatinine value of 1.5 mg/dl as end point. To this end, we assessed the extent of the deterioration of renal function. In the 25 patients who reached the predefined end point of renal death, serum creatinine had increased by an average of 46% from 1.15 ± 0.2 to 1.65 ± 0.24 mg/dl. Calculated creatinine clearance (Cockcroft and Gault formula) was 76 ± 22 ml/min per 1.73 m^2 at baseline and 52 ± 13 ml/min per 1.73 m^2 at the end point. The absolute decrease in creatinine clearance averaged 45 ml/min per 1.73 m^2/year. For comparison, in the nonfailure group, the average change of calculated creatinine clearance was 1.7 ml/min per 1.73 m^2/year.

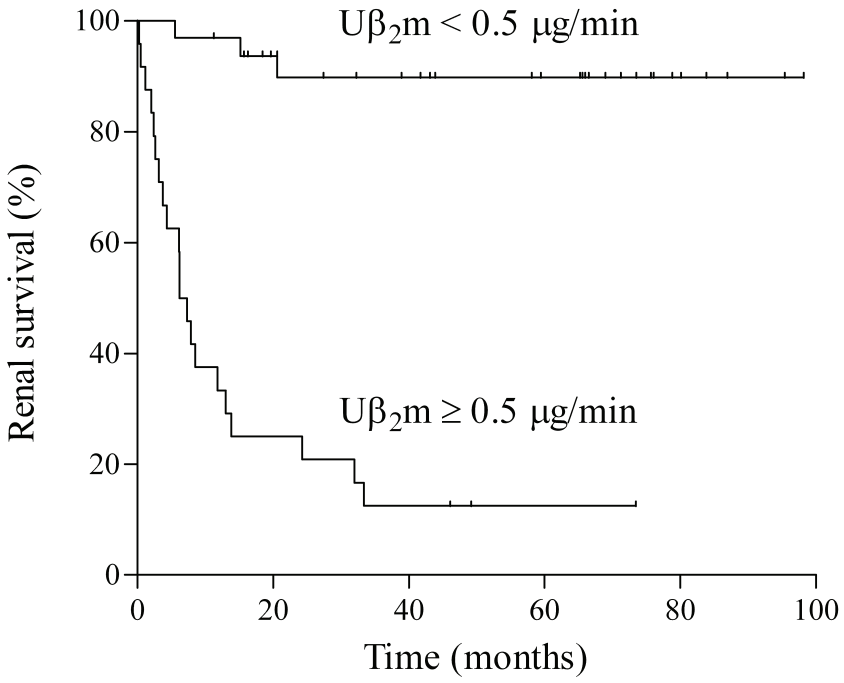


Figure 1. Renal survival in patients with idiopathic membranous nephropathy (iMN) with urinary β_2-microglobulin excretion ($U\beta_2m$) < 0.5 μg/min and ≥ 0.5 μg/min.

Renal death was defined as an increase of serum creatinine to values > 1.5 mg/dl or an increase of serum creatinine > 50%.

When we estimate GFR using the recently developed MDRD formula, the severity of renal dysfunction is even more manifest: The MDRD GFR at the predefined end point (and thus at the start of immunosuppressive therapy) was 37 ± 9 ml/min per 1.73 m^2. Of note, because we did not calibrate serum creatinine values against the standard of the MDRD reference laboratory, our calculated MDRD GFR may underestimate true GFR by 5 ml/min per 1.73 m^2.

The difference in course of renal function between patients with high and low $U\beta_2m$ can be appreciated by comparing the slopes of 1/serum creatinine: In patients with low $U\beta_2m$, the slope was –0.012 dl/mg per year (interquartile range, –0.04 to 0.014); in patients with high $U\beta_2m$, the slope was –0.42 dl/mg per year (interquartile range, -0.91 to -0.16; $P < 0.01$).

A subgroup analysis limited to 44 patients with an initial serum creatinine < 1.2 mg/dl resulted in similar conclusions: Renal survival was 93% at 6 months, 79% at 1 year, and 67% at 3 years. In this subgroup, 14 (32%) patients reached the end point of renal death; at baseline, their serum creatinine was 1.00 ± 0.14 mg/dl and increased by 63% to 1.64 ± 0.31 mg/dl before start of immunosuppressive therapy. In this subgroup analysis, both $U\beta_2m$ and

UIgG predicted prognosis. Renal survival was 33% at 1 year in patients with high Uβ_2m and 97% in patients with low Uβ_2m. Calculated sensitivity and specificity were 79 and 97% for the Uβ_2m and 79 and 90% for the IgG excretion. The specificity improved to 100% when the β_2m and IgG excretion were combined.

We also explored our data using all available parameters. In the initial multivariate analysis, α_1-microglobulin was not included in view of the very high correlation between Uβ_2m and urinary α_1-microglobulin. In univariate analysis, the following parameters were significantly related to renal outcome: serum creatinine ($P < 0.001$), serum albumin ($P < 0.001$), ECC ($P < 0.01$), proteinuria ($P < 0.001$), selectivity index ($P < 0.001$), and urinary excretion of albumin, β_2m, α_1-microglobulin, transferrin and IgG (all $P < 0$ .001). Multivariate analysis revealed that Uβ_2m was the strongest independent predictive factor (relative risk, 1.030; 95% confidence interval, 1.017 to 1.043; $P < 0.001$), indicating that the risk for renal insufficiency increased by 3.0% for every 0.1 µg/min increase of Uβ_2m. After Uβ_2m, serum albumin was identified as the second independent predictive factor (relative risk, 0.786; 95% confidence interval, 0.691 to 0.894; $P < 0.01$). We calculated sensitivity and specificity for the various parameters (Table 2). When combining parameters, specificity can be somewhat increased (Table 2). ROC curves, as depicted in Figure 3, confirmed the best performance of Uβ_2m, as reflected by the AUC.

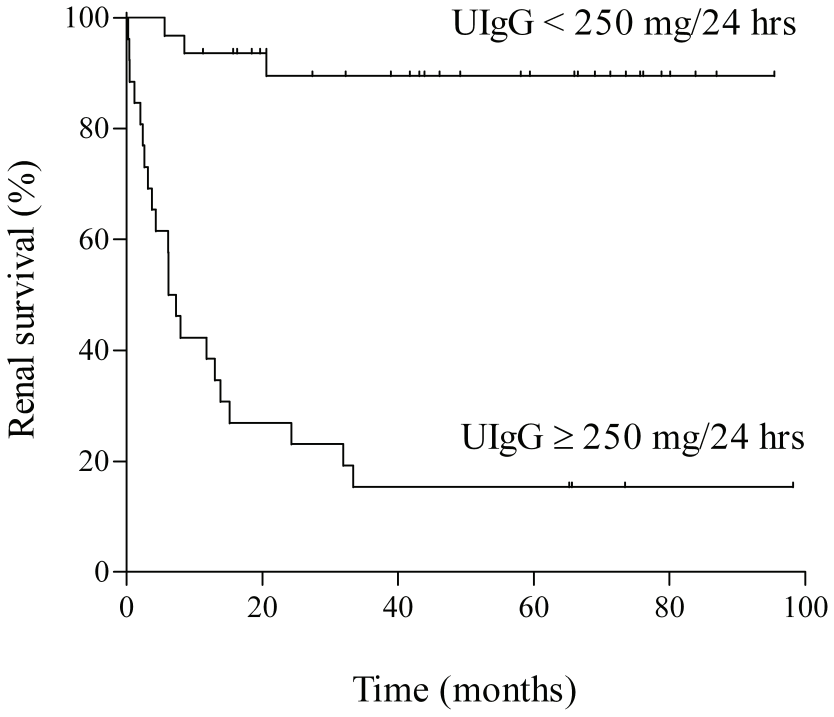


Figure 2. Renal survival in patients with idiopathic membranous nephropathy (iMN) and an IgG excretion < 250 mg/24 h *versus* patients with an IgG excretion ≥250 mg/24 h.

Table 2. Sensitivity, specificity, PPV, and NPV of the most discriminative threshold levels of urinary proteins and creatinine clearance in the prediction of renal failure

Parameter	AUC	Threshold	Sensitivity	Specificity	PPV	NPV
Uβ_2m	0.947	0.5 µg/min	88%	91%	88%	91%
UIgG	0.876	250 mg/24 h	88%	88%	85%	90%
Uα_1m	0.956	40 µg/min	84%	94%	91%	88%
Uexc albumin	0.896	2.8 mg/min	92%	69%	70%	92%
Uexc transferrin	0.906	350 µg/min	80%	84%	80%	84%
Proteinuria	0.898	8 g/24 h	88%	72%	71%	89%
SI	0.687	0.16	76%	50%	54%	73%
ECC 24 h	0.741	80 ml/min per 1.73 m^2	64%	81%	73%	74%
Serum creatinine	0.833	1 mg/dl	76%	81%	76%	81%
Serum albumin	0.913	2.2 g/dl	80%	97%	95%	86%
Combinations:						
high Uβ_2m + high UIgG		0.5 µg/min + 250 mg/24 h	83%	97%	95%	89%
high Uβ_2m + low serum albumin		0.5 µg/min + 2.2 g/dl	75%	100%	100%	84%
high Uα_1m + high UIgG		40 µg/min + 250 mg/24 h	76%	94%	91%	83%
high Uα_1m + low serum albumin		40 µg/min + 2.2 g/dl	72%	100%	100%	82%

Uexc, urinary excretion; β_2m, β_2-microglobulin; α_1m, α_1-microglobulin; SI, selectivity index; PPV, positive predictive value; NPV, negative predictive value.

We specifically evaluated urinary α_1-microglobulin excretion in comparison with β_2m excretion. There was a high correlation between these parameters ($r = 0.80$, $P < 0.001$). In fact, it is evident from Table 2 and Figure 3 that urinary α_1-microglobulin excretion and Uβ_2m give comparable results.

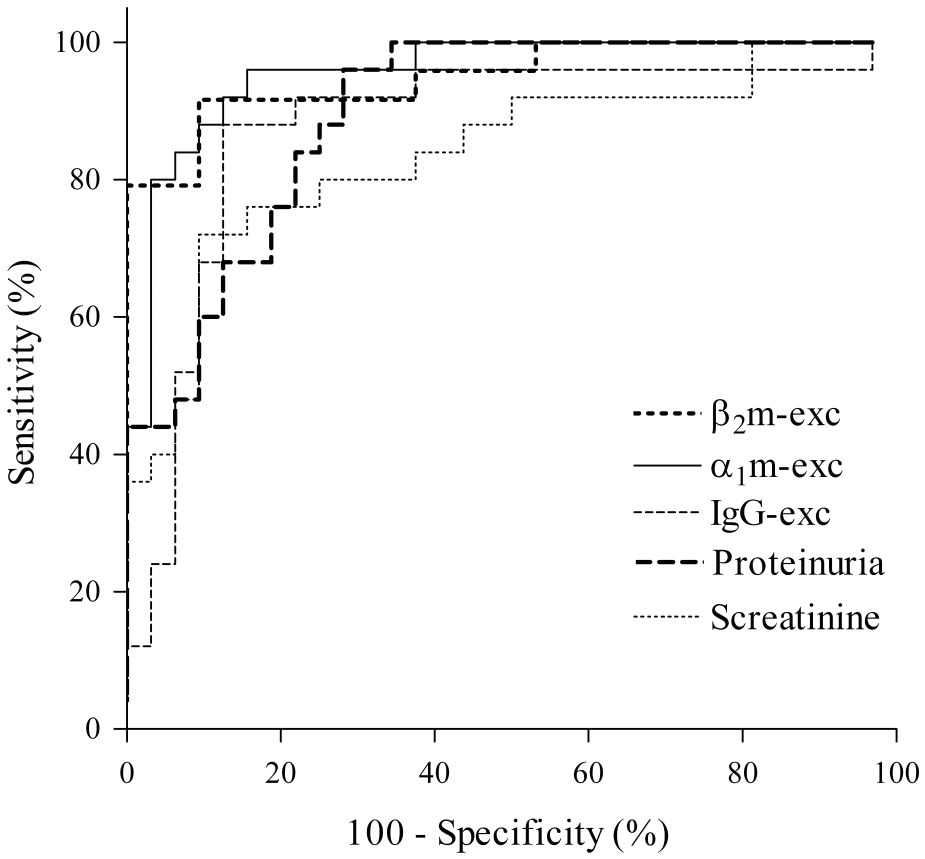


Figure 3. Comparative efficacy of serum creatinine and the urinary excretion of several proteins for predicting renal death in patients with iMN (*n*=57).

Receiver operating characteristics curves of β_2m excretion (β_2m-exc; area under the curve [AUC], 0.947), α_1-microglobulin excretion (α_1m-exc; AUC, 0.956), IgG excretion (IgG-exc; AUC, 0.876), proteinuria per day (AUC, 0.898), and serum creatinine concentration (Screatinine; AUC, 0.833).

Discussion

We have validated the performance of $U\beta_2m$ and UIgG as predictors for renal insufficiency in patients with iMN. To this end, we tested the threshold values developed in our previous studies in a new, prospectively studied patient cohort. Our data clearly demonstrate that $U\beta_2m$ and UIgG predict with high accuracy renal outcome in patients with iMN. In fact, the calculated sensitivities and specificities are nearly identical to the values obtained in our previous studies.[13] Thus, our data indicate that the model parameters are robust.

Our study may be criticized because we used a fixed value of serum creatinine of 1.5 mg/dl as end point for defining renal death. However, it is evident from calculated creatinine clearance and MDRD GFR that renal function was severely disturbed at the end point. The slope of 1/serum creatinine proved that there was a clear loss of renal function. Adopting a doubling of serum creatinine or 50% decrease in GFR as end point would have resulted in even longer withholding of immunosuppressive treatment.

We used a restrictive treatment policy in our patients, initiating immunosuppressive treatment as renal failure was evident. On the basis of the results of the randomized study conducted by Ponticelli *et al*.,[4] one might ask whether delay of treatment is justified especially in patients with a nephrotic syndrome. Our treatment policy was based on our preliminary findings that immunosuppressive treatment with cyclophosphamide is effective in patients with established renal failure. We recently extended these observations and also demonstrated that a restrictive treatment policy results in excellent patient and renal survival rates.[9,12]

In our previous study, we noted that the UIgG was the only variable that was independently associated with renal function deterioration. This superiority of UIgG over $U\beta_2m$ was explained by one patient in whom results of UIgG and $U\beta_2m$ did not concur. In this patient, who developed renal insufficiency, UIgG exceeded the threshold value of 250 mg/day whereas $U\beta_2m$ was below the threshold.[16] In our present, larger study cohort, $U\beta_2m$ was the most significant independent predictive factor. It has been well established that $U\beta_2m$ reflects the severity of tubulointerstitial injury.[19,20] Thus, our findings are in good agreement with studies that have unequivocally shown that in patients with glomerular diseases, renal outcome is more related to the presence and the extent of tubulointerstitial injury than to glomerular pathology. In general, there was a good agreement between $U\beta_2m$ and UIgG. When both parameters were combined, specificity even increased to a value of 97%.

How can we explain that UIgG and Uβ_2m accurately predict renal failure? We propose that UIgG reflects the severity of glomerular damage, whereas Uβ_2m is a marker of tubulointerstitial injury. It has been suggested that IgG or other high molecular weight proteins cause tubular cell activation or injury that results in tubulointerstitial inflammation, the final step toward renal insufficiency.

Thus far, only one model for the identification of patients who have iMN and are at risk for the development of chronic renal failure has been validated. The model was developed with data derived from the Toronto Glomerulonephritis Registry. In the first study, the duration and the level of proteinuria proved to be fairly accurate predictive factors. The best performance was found using a level of proteinuria > 8 g/day for > 6 months. Calculated sensitivity was 66%, and specificity was 88%.[13,14] In the validation study, roughly similar figures were reported with a sensitivity of 58% and a specificity of 93%.[21] In addition, the Toronto group extended the model by calculating a risk score on the basis of the data of a selected 6-months interval with the worst sustained proteinuria. In this model are included the minimum amount of proteinuria in that 6-months interval, the initial creatinine clearance, and the slope of the creatinine clearance during the 6-months period. The risk score model was validated in three different populations and proved quite good with sensitivities varying from 60 to 89%, specificities from 86 to 92%, and an overall accuracy of 79 to 87%.[21] Obviously, this model has a very good performance. However, there are several disadvantages, particularly the need to have an observational period that exceeds a period of 6 months and the necessity of multiple, accurate 24-h urine collections. Our model is based on the collection of a single timed urine sample collected in the morning period.

Furthermore, it is unproved whether the Toronto model can be applied to patients with newly diagnosed iMN. The model has been validated and applied to a group of patients with well-defined follow-up. This suggests that a long observation period was used to define the 6-months period with the worst sustained proteinuria. In more than one quarter of the patients, the 6-months period started > 12 months after renal biopsy. Therefore, the model may not be applicable to patients with a follow-up after biopsy of < 12 to 18 months.

In the present study, we specifically analysed the value of urinary α_1-microglobulin, a low molecular weight protein like β_2m. In routine clinical practice, measurement of urinary α_1-microglobulin is easier in view of its relative stability at pH < 6.0. We observed a very high correlation between Uβ_2m and urinary α_1-microglobulin. Sensitivities and specificities were also comparable, although, admittedly, the threshold values used for α_1-microglobulin should

be validated in a second population. Our data confirm and strengthen the conclusion of Bazzi *et al*.[22] In a small cohort of 19 untreated patients with iMN, a nephrotic syndrome, and normal renal function, these authors found that urinary α_1-microglobulin predicted the development of chronic renal failure with a sensitivity and specificity of 100%. We have applied their threshold value of 33.5 mg/g creatinine to our study cohort of 57 patients and calculated a sensitivity of 88% and a specificity of 78%. Bazzi *et al.* also reported the predictive value of UIgG. Using a threshold value of 110 mg/g creatinine, sensitivity was 100% and specificity was 69%. Applying this threshold value to our study cohort, we calculated a sensitivity of 92% and a specificity of 63 %. We used a higher cut-off value (250 mg/day, approximately 180 mg/g creatinine), thereby increasing specificity. We believe that a high specificity should be pursued to be able to avoid unnecessary immunosuppressive therapy in patients with iMN.
The data of our secondary analysis suggest that serum albumin may have added value as a prognostic marker. Admittedly, this needs confirmation in another patient cohort. Can we avoid unnecessary immunosuppressive treatment by using Uβ_2m and UIgG as prognostic markers? From our data, it can be calculated that when used in the present population with a failure rate of 44% (which is in close agreement with literature data), our established threshold values would have resulted in the unnecessary treatment of one patient (1.8% overall, 4.8% of all treated patients), whereas 31 patients rightly would not have received treatment.

Conclusion

We have validated the performance of Uβ_2m and UIgG as prognostic markers in patients with iMN. Urinary α_1-microglobulin can replace Uβ_2m. Use of these markers will allow identification of high-risk patients at an early stage. We propose that these markers may help to guide the time of start of immunosuppressive treatment in individual patients.

Acknowledgments

A.J.W. Branten is supported by a grant from the Dutch Science Foundation (NWO-MW 920-03-038).

We thank Drs. J.J. Beutler and J.L.J. Jansen, Jeroen Bosch Hospital, 's-Hertogenbosch; Dr. P.A. Bleeker, Hospital Rivierenland, Tiel; Dr. M.A.G.J. ten Dam, Canisius Wilhelmina Hospital, Nijmegen; Dr. P.G.G. Gerlag, Máxima Medical Center, Veldhoven; Drs. W. Grave and J.J.J.M. Wirtz, St. Laurentius Hospital, Roermond; Dr. M. den Hartog, Hospital Gelderse Vallei, Ede; Dr. L.H. Hulsteyn, Bernhoven, Veghel; Drs. R. van Leusen and K.J. Parlevliet, Hospital Rijnstate, Arnhem; Dr. A.J. Luik, Hospital VieCuri, Venlo; Drs. T.C. Noordzij and H.P.C. van Roermund, St. Franciscus Hospital, Roosendaal; Dr. J.J.G.Offerman, Isala Klinieken, Zwolle; Dr. P.L. Rensma, St. Elisabeth Hospital, Tilburg; Dr. G. Schrijver, Hospital Rode Kruis, Beverwijk; Dr. R.E.H. Smeets, St. Anna Hospital, Geldrop; Dr. P.F.M.J. Spooren, TweeSteden Hospital, Tilburg; and Dr. P.J. Stijnen, Amphia Hospital Breda, for participation in this study.

References

1. Haas M, Meehan SM, Karrison TG, Spargo BH. Changing Etiologies of unexplained adult nephrotic syndrome: A comparison of renal biopsy findings from 1976-1979 and 1995-1997. *Am J Kidney Dis* 1997; 30: 621-631
2. Donadio JJV, Torres VE, Velosa JA, *et al*. Idiopathic membranous nephropathy: The natural history of untreated patients. *Kidney Int* 1988; 33: 708-715
3. Schieppati A, Mosconi L, Perna A, *et al*. Prognosis of untreated patients with idiopathic membranous nephropathy. *New Engl J Med* 1993; 329: 85-89
4. Ponticelli C, Zuchelli P, Passerini P, *et al*. A 10-year follow-up of a randomized study with methylprednisolone and chlorambucil in membranous nephropathy. *Kidney Int* 1995; 48: 1600-1604
5. Muirhead N. Management of idiopathic membranous nephropathy: evidence-based recommendations. *Kidney Int* 1999; 55 (Suppl. 70): S47-S55
6. Cattran DC. Idiopathic membranous glomerulonephritis. *Kidney Int* 2001; 59: 1983-1994
7. Davison AM, Cameron JS, Kerr DN, *et al*. The natural history of renal function in untreated idiopathic membranous glomerulonephritis in adults. *Clin Nephrol* 1984; 22: 61-67
8. Honkanen E, Tornroth T, Gronhagen-Riska C, Sankila R. Long-term survival in idiopathic membranous glomerulonephritis: can the course be clinically predicted? *Clin Nephrol* 1994; 41: 127-134

9. du Buf-Vereijken PWG, Feith GW, Hollander D, *et al*. Restrictive use of immunosuppressive treatment in patients with idiopathic membranous nephropathy: high renal survival in a large patient cohort. *Q J Med* 2004; 97: 353-360

10. Branten AJW, Reichert LJM, Koene RAP, Wetzels JFM. Oral cyclophosphamide versus chlorambucil in the treatment of patients with idiopathic membranous nephropathy and renal insufficiency. *Q J Med* 1998; 91: 359-366

11. Mathieson PW, Turner AN, Maidment CG, *et al*. Prednisolone and chlorambucil treatment in idiopathic membranous nephropathy with deteriorating renal function. *Lancet* 1988; 2: 869-872

12. du Buf-Vereijken PWG, Branten AJW, Wetzels JFM, for the Membranous Nephropathy Study Group. Cytotoxic therapy for membranous nephropathy and renal insufficiency; improved renal survival but high relapse rate. *Nephrol Dialysis Transplant* 2004; 19: 1142-1148

13. Reichert LJM, Koene RAP, Wetzels JFM. Prognostic factors in idiopathic membranous nephropathy. *Am J Kidney Dis* 1998; 31: 1-11

14. Pei Y, Cattran DC, Greenwood C. Predicting chronic renal insufficiency in idiopathic membranous glomerulonephritis. *Kidney Int* 1992; 42: 960-966

15. Reichert LJM, Koene RAP, Wetzels JFM. Urinary excretion of β_2-microglobulin predicts renal outcome in patients with idiopathic membranous nephropathy. *J Am Soc Nephrol* 1995; 6: 1666-1669

16. Reichert LJM, Koene RAP, Wetzels JFM. Urinary IgG excretion as a prognostic factor in idiopathic membranous nephropathy. *Clin Nephrol* 1997; 48: 79-84

17. Jacobs EMG, Vervoort G, Branten AJW, *et al*. Atrial natriuretic peptide increases albuminuria in type I diabetic patients: evidence for blockade of tubular protein reabsorption. *Eur J Clin Invest* 1999; 29: 109-115

18. Levey AS, Bosch JP, Breyer Lewis J, *et al*. for the Modification of Diet in Renal Disease Study Group. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. *Ann Intern Med* 1999; 130: 461-470

19. Lai KN, Mac-Moune Lai F, Vallance-Owen J. The clinical use of serum β2-microglobulin and fractional β2-microglobulin excretion in IgA nephropathy. *Clin Nephrol* 1986; 25: 260-263

20. Portman RJ, Kissane JM, Robson AM, *et al*. Use of β2-microglobulin to diagnose tubulo-interstitial renal lesions in children. *Kidney Int* 1986; 30: 91-98

21. Cattran DC, Pei Y, Greenwood CMT, *et al*. Validation of a predictive model of idiopathic membranous nephropathy: its clinical and research implications. *Kidney Int* 1997; 51: 901-907

22. Bazzi C, Petrini C, Rizza V, *et al*. Urinary excretion of IgG and α1-microglobulin predicts clinical course better than extent of proteinuria in membranous nephropathy. *Am J Kidney Dis* 2001; 38: 240-248

Chapter 7

Measurement of β_2-microglobulin in urine: utility of a single dose of acetazolamide

Peggy W.G. du Buf-Vereijken[1,3],
Ina S. Klasen[2], Gertrude van de Wiel[2],
and Jack F.M. Wetzels[1]

[1]Department of Nephrology, and [2]Department of Clinical Chemistry,
Radboud University Nijmegen Medical Center, Nijmegen,
[3]Department of Internal Medicine, Amphia Hospital, Breda,
The Netherlands

Submitted

Abstract

The urinary excretion of β_2-microglobulin (β_2m) is used as a marker of renal tubulo-interstitial injury. Unfortunately, β_2m is rapidly degraded in acid urine (pH < 6.0). We questioned if the use of a single dose of the carbonic anhydrase inhibitor acetazolamide would allow valid measurement of urinary β_2m without influencing its excretion rate.

We have studied 10 healthy volunteers (4 male, 6 female) who received no drug (N), sodium bicarbonate (B) or acetazolamide (A) orally on three separate days. Timed 2 hour urine samples were collected. In addition, we have studied 11 patients (all male) with proteinuric renal disease, mean serum creatinine of 117 μmol/l and mean proteinuria of 3.3 g/10 mmol creatinine. After pretreatment with sodium bicarbonate and baseline measurements acetazolamide was given and another urine sample was collected.

The use of acetazolamide increased urinary pH and allowed measurement of urinary β_2m in all volunteers and patients. When compared with sodium bicarbonate, use of acetazolamide did not influence the urinary excretion of β_2m. Side effects were frequent and included paresthesias, change in taste and dizziness.

In conclusion, administration of a single dose of acetazolamide (with or without pretreatment with sodium bicarbonate) increases urinary pH and does not influence urinary excretion rate of β_2m. Use of acetazolamide thus allows valid measurement of urinary β_2m.

Introduction

Beta-2-microglobulin (β_2m) is a low-molecular-weight (LMW) protein (Molecular mass 11.8 kD) that is readily filtered through the glomerulus and almost completely reabsorbed by the proximal tubules.[1] An increased urinary excretion of β_2m (UEβ_2m) is a good marker of tubulo-interstitial injury.[2-4] We have previously demonstrated that measurement of urinary excretion of β_2m allows to predict prognosis in patients with membranous nephropathy with high sensitivity and specificity.[5] Unfortunately, β_2m is rapidly degraded in acid urine (pH < 6.0),[6-8] a process which already occurs during retention of the urine in the bladder.[7;8]

As an alternative, the low molecular weight proteins α_1-microglobulin (α_1m) and Retinol Binding Protein (RBP) can be used. However, both α_1m and RBP are somewhat unstable at urinary pH below 7.0, and for the measurement of these proteins also alkalinization of urine has been recommended.[7] Furthermore, in serum both α_1m and RBP are bound to proteins whereas β_2m is only present in its free form, thus freely filterable. The fractional excretion of β_2m thus quantitatively reflects tubular reabsorptive capacity. In contrast, the urinary excretion of α_1m and RBP is not only dependent on tubular reabsorption but is also influenced by changes in glomerular permeability.[9] Quantitatively, proximal tubular reabsorption can therefore best be assessed by measuring fractional excretion of β_2m.[10]

To ensure urine alkalinization we thus far have prescribed oral sodium bicarbonate, 4 g in the evening and 2 g in the morning before collection of a timed (two hours) urine sample. However, even with this dose of sodium bicarbonate, urinary pH (UpH) was below 6.0 in 7% of 944 consecutive urine samples collected in patients with proteinuria (unpublished data). Acetazolamide is a diuretic agent that inhibits carbonic anhydrase activity, thus increasing urinary bicarbonate excretion and UpH. We questioned if the administration of a single dose of acetazolamide (partly after pretreatment with sodium bicarbonate) would allow valid measurement of β_2m in the urine, without influencing protein excretion rates.

Materials and Methods

We first have studied 10 healthy volunteers (4 male, 6 female) without proteinuria and with normal renal function (median serum creatinine 80 µmol/l, range 67-92 µmol/l). In these volunteers timed urine samples were collected in the morning at three consecutive days. In

random order the volunteers received nothing, sodium bicarbonate (4 g orally the evening before and 2 g in the morning about 4 hours before urine sampling) or acetazolamide (250-500 mg orally 2 hours before urine sampling). Blood pressure was measured with an automatic device.

We next studied eleven patients (11 male) with proteinuria. Mean serum creatinine was 117 µmol/l (range 91-285 µmol/l) and proteinuria amounted 3.3 g/10 mmol creatinine (range 0.8-18.7 g/10 mmol creatinine). These patients were studied according to our standard protocol for patients with proteinuria, i.e. timed urine samples were collected in the morning after an overnight fast and after pretreatment with sodium bicarbonate. At the end of the urine collection period, acetazolamide 250 mg orally (n=9) or i.v. (n=2) was given and a second urine sample was collected two hours later. Blood pressure was measured at regular intervals during the collection period with an automatic device (DINAMAP, Criticon, Tampa FI, USA).

In the urine samples pH, creatinine, β_2m, albumin and sodium were measured.

Measurement of creatinine was done with the modified Jaffe technique on a Hitachi 747 auto analyser (Roche, Almere, The Netherlands), β_2m and albumin were measured by ELISA[11] and pH with a Checker ®2 (Hanna Instruments, IJsselstein, The Netherlands). Urinary β_2m excretion was not measured in samples with UpH < 6.0.

For statistical analysis the Wilcoxon Signed Ranks Test was used for paired data. Values are given as means ± SD or medians (range) when appropriate.

Results

Overall results are given in Table 1.

Changes in the urinary excretion of β_2m for each individual are depicted in Figure 1.

In the healthy volunteers administration of acetazolamide resulted in significantly higher UpH. Use of acetazolamide enabled measurement of β_2m in all urine samples. In contrast, measurement of β_2m in urine was not possible in four urine samples without pretreatment and in two urine samples after sodium bicarbonate. Mean arterial blood pressure and urinary excretion of albumin were not different after acetazolamide when compared with no

pretreatment or sodium bicarbonate. We also observed no significant differences in the urinary excretion of β_2m when comparing acetazolamide and sodium bicarbonate.

In the patients acetazolamide likewise increased UpH. In these patients, who were on a sodium-restricted diet and were regularly using diuretics, acetazolamide induced a fall in mean arterial pressure (MAP).

Table 1. Effects of acetazolamide on urinary pH and urinary excretion of β_2m and albumin

	Study I: Healthy volunteers (n=10)			Study II: Proteinuria patients (n=11)	
	No pretreatment	Bicarbonate	Acetazolamide	Bicarbonate	Plus Acetazolamide
UpH	6.21 (4.97-7.39)	7.57 (5.80-7.89)	8.0** (7.91-8.26)	7.08 (5.48-7.90)	7.80** (6.38-8.40)
Nr of subjects with UpH < 6.0	4	2	0	2	0
UEβ_2m [#] (mg/10 mmol creatinine)	0.081 (0.047-0.117)	0.087 (0.077-0.141)	0.108 (0.063-0.150)	1.63 (0.47-7.67)	1.36 (0.26-6.87)
UEalb (mg/10 mmol creatinine)	7.31 (3.96-13.60)	8.67 (4.33-20.83)	8.28 (4.13-12.67)	2655 (939-14,129)	2047* (635-14,200)
MAP (mm Hg)	91 (71-98)	93 (82-109)	93 (74-106)	101 (88-116)	87** (63-105)

Values are given as medians with range.
UpH, urinary pH; UEβ_2m, urinary excretion of β_2-microglobulin; UEalb, urinary excretion of albumin; MAP, mean arterial pressure. [#]For UEβ_2m values are given for 8 controls and 9 patients respectively, for whom paired samples were available that allowed a comparison between sodium bicarbonate and acetazolamide.
* $P < 0.05$; ** $P < 0.01$ for acetazolamide compared with bicarbonate.

Overall, urinary excretion of β_2m was not significantly different. In contrast, we observed a significant decrease in urinary albumin excretion after acetazolamide (P = 0.033). This decrease in albuminuria correlated with the change in MAP (r = 0.594, P = 0.05).

If we compare the values of urinary excretion of β_2m after acetazolamide and after sodium bicarbonate it is evident that there is no significant difference (Figure 1). The percentage difference between UEβ_2m after acetazolamide and sodium bicarbonate amounted median -4% (-20 – 56%) for the volunteers and -17% (-82 – 20%) for the patients.

After the administration of acetazolamide side effects occurred frequently, mainly consisting of paresthesias (58%), changes in taste (32%) and dizziness (29%). Although these side

effects were not severe, some individuals expressed their reluctance to use acetazolamide again.

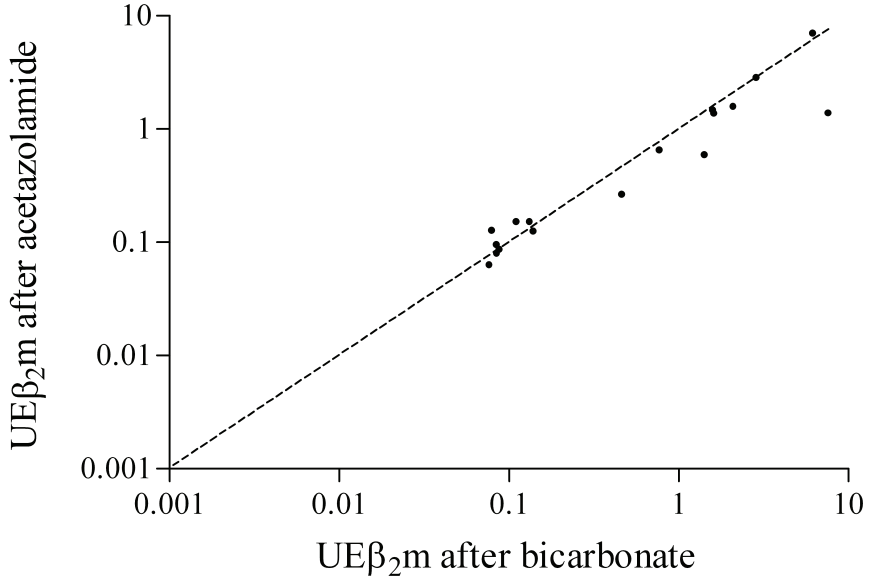


Figure 1. Correlation between the urinary excretion of β_2m (β_2m, in mg/10 mmol creatinine, logarithmic scale) after sodium bicarbonate and acetazolamide in volunteers and patients. The reference line is the line of identity.

Discussion

Administration of acetazolamide adequately increased urinary pH, thus allowing measurement of urinary β_2m. Acetazolamide did not itself influence the urinary excretion of β_2m. One might have expected an increased urinary excretion of albumin and/or β_2m since acetazolamide increases proximal tubular flow rate. In the healthy volunteers, no changes in urinary excretion of β_2m or albumin occurred. In the patients, we observed a numerical decrease in urinary β_2m excretion and a significant decrease in albuminuria. This however could readily be explained by the decrease in blood pressure that we observed, a likely consequence of the administration of acetazolamide to sodium-restricted patients who were using diuretics and ACE-inhibitors.

We observed frequent side effects even with the use of only a single dose of acetazolamide. These side effects are well known and may limit the frequent use of acetazolamide in clinical practice.

A reasonable strategy would be to use acetazolamide only in those patients in whom a urinary pH above 6.0 cannot be achieved with the administration of sodium bicarbonate.

References

1. D'Amico G, Bazzi C. Pathophysiology of proteinuria. *Kidney Int* 2003; 63: 809-825
2. Portman RJ, Kissane JM, Robson AM. Use of β_2-microglobulin to diagnose tubulo-interstitial renal lesions in children. *Kidney Int* 1986; 30: 91-98
3. Tomlinson PA, Dalton RN, Hartley B, *et al.* Low molecular weight protein excretion in glomerular disease: a comparative analysis. *Pediatr Nephrol* 1997; 11: 285-290
4. Guder WG, Ivandic M, Hofmann W. Physiopathology of proteinuria and laboratory diagnostic strategy based on single protein analysis. *Clin Chem Lab Med* 1998; 36: 935-939
5. Reichert LJ, Koene RA, Wetzels JF. Urinary excretion of β_2-microglobulin predicts renal outcome in patients with idiopathic membranous nephropathy. *J Am Soc Nephrol* 1995; 6: 1666-1669
6. Davey PG, Gosling P. β_2-Microglobulin instability in pathological urine. *Clin Chem* 1982; 28: 1330-1333
7. Donaldson MD, Chambers RE, Woolridge MW, Whicher JT. Stability of α_1-microglobulin, β_2-microglobulin and retinol binding protein in urine. *Clin Chim Acta* 1989; 179: 73-77
8. Blumsohn A, Morris BW, Griffiths H, Ramsey CF. Stability of β_2-microglobulin and retinol binding protein at different values of pH and temperature in normal and pathological urine. *Clin Chim Acta* 1991; 195: 133-137
9. Hofmann W, Edel H, Guder WG. A mathematical equation to differentiate overload proteinuria from tubulo-interstitial involvement in glomerular diseases. *Clin Nephrol* 1995; 44: 28-31
10. Ten Dam MA, Branten AJ, Klasen IS, Wetzels JF. The gelatin-derived plasma substitute Gelofusine causes low-molecular-weight proteinuria by decreasing tubular protein reabsorption. *J Crit Care* 2001; 16: 115-120
11. Jacobs EM, Vervoort G, Branten AJ, *et al*. Atrial natriuretic peptide increases albuminuria in type I diabetic patients: evidence for blockade of tubular protein reabsorption. *Eur J Clin Invest* 1999; 29: 109-115

Chapter 8

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

Peggy W.G. du Buf-Vereijken[1,2], Jack F.M. Wetzels[2]

[1]Department of Internal Medicine, Amphia Hospital, Breda,
[2] Department of Nephrology, Radboud University Nijmegen Medical Centre, Nijmegen,
The Netherlands

Nephrol Dial Transplant 2005; in press in revised form

Abstract

Background. In patients with idiopathic membranous nephropathy (iMN) an increased urinary excretion of high (IgG) and low (β_2-microglobulin (β_2m), α_1-microglobulin (α_1m)) molecular weight proteins predicts prognosis and precedes renal insufficiency. We have studied the changes in the urinary excretion of these proteins in patients with iMN 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, β_2m, and α_1m 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 in glomerular permselectivity and tubular protein reabsorption within 2 months after start of therapy. Despite a partial remission of proteinuria within 12 months in most patients, evidence of tubulo-interstitial injury remained apparent. Levels of urinary IgG, β_2m or α_1m neither at baseline nor at 12 months clearly predicted the occurrence of a remission or a relapse to nephrotic range proteinuria. In case of a persistent stable remission we observed a gradual decrease in urinary β_2m towards normal values.

Conclusions. In patients with iMN and renal insufficiency treatment with cyclophosphamide and steroids resulted in an improvement in 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.

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 β_2-microglobulin (β_2m) and α_1-microglobulin (α_1m) accurately predict prognosis in patients with idiopathic membranous nephropathy and normal renal function.[5-8] The reported data support the following sequence of events that lead to renal failure: severe alterations in glomerular permselectivity (identified by non-selective proteinuria[9] and high levels of urinary IgG) are followed by tubulo-interstitial injury (identified by high levels of urinary β_2m and α_1m), which ultimately causes renal insufficiency.[10-12] In our study[7] in multivariate analysis urinary β_2m 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 over 80% of patients developed a partial remission of proteinuria. Unfortunately, relapses occurred in 28% of patients after five 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 predicting 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

bodyweight/day for 12 months and steroids. The corticosteroid regimen consisted of three consecutive 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 bodyweight on alternate days for six months. In patients treated most recently, standardized measurements of urinary proteins and renal function were performed at 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-hour urine samples for measurement of creatinine and total protein. 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 4 g of oral sodium bicarbonate in the evening before, and additionally 2-4 g at arrival in the ward to achieve an urinary pH > 6.0, which is necessary to allow reliable measurements of urinary β_2m. Two hours before arrival in the hospital, 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] At arrival at the ward, up to 500 ml tap water was given to enforce diuresis. The patients remained supine during two hours except for voiding. Blood pressure measurements were done using an automated device (DINAMAP, Criticon, Tampa FI, USA), with 6 consecutive readings registered every 5 minutes after 10 minutes rest; these readings were used to calculate the average MAP. The timed urine sample, collected after two hours, was used for the measurement of urinary pH, β_2m, α_1m, IgG, transferrin, albumin, total protein and creatinine. Only in urine with a urinary pH > 6.0 β_2m excretion was measured. Laboratory parameters were measured in blood samples collected in the mid of the urine collection period.

The use of angiotensin converting enzyme inhibitors and/or angiotensin-II-type 1 receptor antagonist as well as of HMG-CoA-reductase inhibitors (statins) during the treatment year was noted.

Laboratory measurements

Serum creatinine and cholesterol, and urinary total protein and creatinine were measured with standard automated techniques. The concentrations of α_1m, 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, Gloostrup, Denmark). Serum and urinary β_2m were measured by ELISA, as described before.[16]

Calculations and statistics

Creatinine clearance (ECC) was calculated according to the formula Ucr x V / Pcr, where Ucr and Pcr 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. The excretion of the low and high molecular weight proteins is expressed per unit of time (min or 24 hours) to allow comparison with our previously reported threshold values: for UIgG 250 mg/24 hr[5;7] for Uβ_2m 500 ng/min (=0.5 µg/min)[6;7] and for α1m 40 µg/min.[7;8]

The Selectivity index (SI) of proteinuria was calculated using the formula: SI = (UIgG/SIgG) * (STransf/UTransf), where U = urine, S = serum, Transf = transferrin. Non-selective proteinuria was defined as a SI ≥ 0.21. The tubular reabsorption of β_2m was calculated using the formula: reabsorption = 1 - fractional excretion (FE), and expressed as a percentage. FE of β_2m = (Uβ_2m/Sβ_2m) / (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 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, USA).

Definitions

A complete remission of proteinuria (CR), partial remission (PR), persistent proteinuria (PP) and nephrotic range proteinuria (NS) 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 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 and 2).

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.

Table 1. 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

	t = 0 months	*t* = 2 months	*t* = 6 months	*t* = 12 months
Serum values				
Screatinine (μmol/l)	152 (132-278)	137* (108-221)	126** (83-168)	128** (89-215)
Sβ_2m (mg/l)	5.32 (3.13-7.52)	3.45** (2.38-5.72)	2.80** (2.20-4.60)	2.91** (1.90-6.46)
Salbumin (g/l)	26 (18-35)	30** (20-38)	36** (32-39)	41** (34-44)
SIgG (g/l)	4.6 (2.4-13.2)	2.6** (1.0-4.8)	3.6** (2.1-10.6)	6.1* (3.3-10.2)
Scholesterol (mmol/l)	6.7 (4.8-18.0)	6.4 (4.1-10.0)	5.3* (4.0-6.3)	4.8** (3.4-6.4)
Calculated creatinine clearances				
24-hours urine (ml/min)	53 (25-68)	59* (29-100)	68** (45-108)	62* (33-113)
2-hours urine (ml/min)	39 (5-52)	52** (21-68)	50** (33-81)	51** (33-87)

* $P < 0.05$, ** $P < 0.01$ of values compared with baseline values
S, serum; β_2m, β_2-microglobulin

All patients used an ACE-inhibitor (*n*=10) or an angiotensin-II-type1 receptor antagonist (*n*=1) at the start and during the treatment year and six patients used a statin. Mean arterial

pressure was 90 (69 – 112) mm Hg at baseline and did not change significantly during the treatment year. Data are given in Tables 1 and 2.

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

	t = 0 months	t = 2 months	t = 6 months	t = 12 months
Proteinuria (g/10 mmol creatinine)	11.7 (6.3-31.3)	5.0** (1.4-9.4)	1.7** (0.7-5.9)	1.0** (0.3-3.9)
Albuminuria (g/10 mmol creatinine)	7.9 (4.9-16.6)	3.4** (0.9-6.4)	1.2** (0.5-3.8)	0.7** (0.1-3.8)
UEβ_2m (n=7)[#] (µg/min)	15.1 (2.5-46.9)	3.2 (0.3-56.8)	1.8 (0.2-37.6)	2.4 (0.2-47.3)
UEα_1m [##] (µg/min)	69.0 (17.0-306.7)	55.1 (17.0-182.2)	30.4* (7.3-126.1)	16.4* (8.2-137.7)
UEIgG (mg/24 hour)	348 (188-787)	61** (5-253)	16** (4-105)	16** (12-124)
Fractional excretion of IgG	0.22 (0.08-0.43)	0.03** (0.00-0.24)	0.006** (0.00-0.08)	0.004** (0.00-0.03)
SI (IgG/Transferrin) (n=6)	0.35 (0.27-0.53)	0.20 (0.12-0.74)	0.22* (0.08-0.42)	
Number of patients in partial remission	0	1	5	10

[#] Normal urinary excretion of β_2m < 0.2 µg/min (< 200 ng/min)
[##] Normal urinary excretion of α_1m < 10 µg/min.
* P < 0.05; ** P < 0.01 of values compared with baseline value
UE, urinary excretion; β_2m, β_2-microglobulin; α_1m, α_1-microglobulin; SI, Selectivity Index

All patients had renal insufficiency as reflected by serum creatinine and calculated creatinine clearances. Creatinine clearances calculated from the 2-hours urine, collected after cimetidine, are significantly lower than creatinine clearances calculated from 24-hours urine, reflecting the inhibition of creatinine secretion by cimetidine. All patients had nephrotic range proteinuria, ranging from 6.3 – 31.3 g/10 mmol creatinine. Renal function improved in all patients. We also observed a reduction of proteinuria in all patients (Table 2). All but one patient developed a partial remission, but no patient developed a complete remission within the first year of treatment. The decrease in proteinuria was paralleled by a significant rise in serum albumin and decrease in serum cholesterol (Table 1). From Table 1 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 start of therapy, most likely the result of the immunosuppressive lymphocytotoxic therapy.

Changes in the urinary excretion of the various low- and high molecular weight proteins can be derived from Table 2. 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, partly explained by the initial decrease in serum IgG. However, the greater reduction of urinary IgG also reflected an improvement in glomerular size-selectivity as indicated by the lower selectivity index. The time course of the urinary excretion of β_2m could only be fully assessed in 7 patients in whom urinary pH was above 6.0 in all measurements. Still, it is evident from Table 2 that we observed a rapid and large reduction in urinary excretion of β_2m. A similar pattern was observed for urinary α_1m, 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.

The data of these repeated measurements have allowed us to study in more detail the possibility of blockade of tubular reabsorption of β_2m by IgG. Based on animal experiments it has been suggested that an increased urinary excretion of β_2m 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. In Figure 1 we have plotted the reabsorption of β_2m against the urinary excretion of IgG (mg/100 ml 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 β_2m excretion ($r^2 = 0.14$). Thus, the urinary excretion of β_2m is a valuable marker of tubular dysfunction and not merely a reflection of glomerular permeability changes.

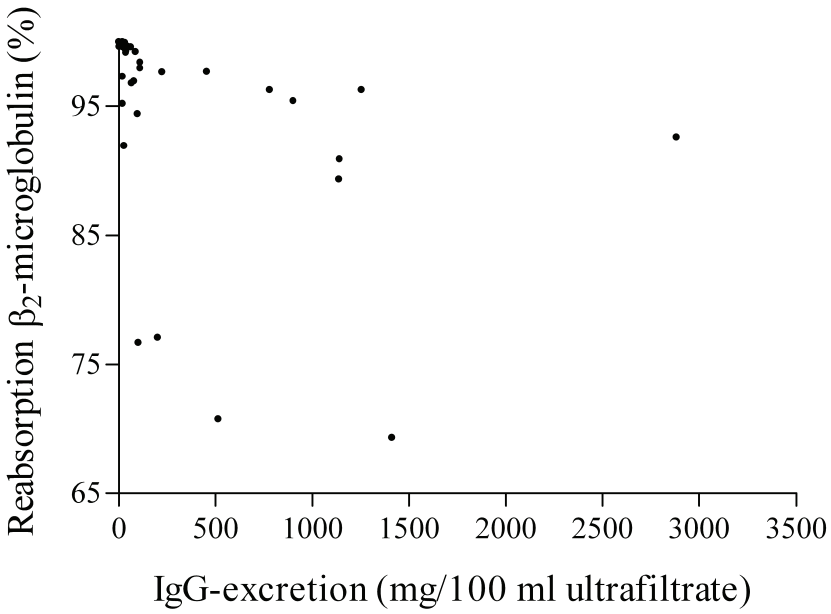


Figure 1. Relation between tubular reabsorption of β_2-microglobulin and the urinary IgG-content.
$r^2 = 0.14$.

Predictive value of tubular proteinuria at the end of the treatment year (Table 3)

Twenty-five patients (21 males, 4 females; age 58 (38-75) years) were studied at the end of the treatment year (12 months). Follow-up from the start of treatment amounted 36 (14-76) months. 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 into a complete remission at 18 (9-22) months after the start of the treatment. In two patients proteinuria improved to values below 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 (1 out of a complete remission, 3 out of a partial remission and 1 out of persistent proteinuria), after 34 (23-45) months. Thus, at the end of follow-up, 18 patients were in remission (3 in complete remission, 15 in partial remission), 1 had persistent proteinuria and 6 patients had a (relapse to) nephrotic syndrome.

Renal function had improved in all patients, from a serum creatinine of 161 (112-444) μmol/l at the start of treatment to a lowest value of 114 (70-255) μmol/l ($P < 0.01$) after a median of

10 months. Serum creatinine amounted 120 (74-255) μmol/l at 12 months and 122 (79-267) μmol/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) has occurred in 2 patients during follow-up, both patients also had a relapse of proteinuria to a nephrotic syndrome. Thus far no patient has developed ESRD.

Values of serum and urine parameters for the 25 patients at the end of the 12-month treatment period are given in Table 3. 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 β_2m and α_1m reached values below the thresholds in eight out of 21 and 16 out of 25 patients respectively.

Table 3. Laboratory parameters and calculated creatinine clearances in 25 patients with a measurement at 12 months

Serum measurements		**Urine measurements**	
Screatinine (μmol/l)	120 (74-255)	Proteinuria (g/10 mmol creat)	1.0 (0.1-13.0)
Sβ_2m (mg/l)	3.2 (1.9-6.5)	UEβ_2m # (μg/min)	1.0 (0.1-47.3)
Salbumin (g/l)	40 (24-44)	Albuminuria (g/10 mmol creat)	0.73 (0.05-7.31)
Scholesterol (mmol/l)	4.8 (3.3-6.7)	UEα_1m (μg/min)	18.6 (6.8-137.7)
SIgG (g/l)	5.6 (3.0-10.2)	UEIgG (mg/24 hour)	16 (4.9-216)
Calculated creatinine clearances			
24-hours urine (ml/min)	67 (31-113)		
2-hours urine (ml/min)	51 (22-93)		

[#] *n*=21 patients

S, serum; UE, urinary excretion; β_2m, β_2-microglobulin; α_1m, α_1-microglobulin

We next evaluated if the urinary excretions of β_2m, α_1m and IgG at 12 months were useful predictors 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 total follow-up time in case no failure occurred, time to relapse in 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 excretions of IgG, β_2m and α_1m, serum creatinine and creatinine clearance and serum albumin. Thus, the measurements at 12 months do not allow to predict treatment failure or relapse.

In a subgroup analysis we have investigated the possibility to predict 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 more than 24 months after the occurrence of the remission, 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 case of a stable remission

Since urinary β_2m excretion was still abnormal in most patients at 12 months, we have continued to study patients during longer follow-up. Thus far, repeated measurements were done in 17 patients (13 male, 4 female; age 58 (38-71) years at start of treatment). These later measurements were done 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 four months after onset of a relapse.

In patients with a stable remission we observed a gradual further decrease in urinary β_2m-excretion (Figure 2). In contrast, in patients with a relapse there was a sharp increase in urinary IgG excretion from 14 to 373 mg/24 hours ($P < 0.05$), in urinary β_2m-excretion from 0.7 to 4.9 μg/min (*n*=3; NS) and in urinary α_1m-excretion from 25.9 to 63.1 μg/min ($P < 0.05$).

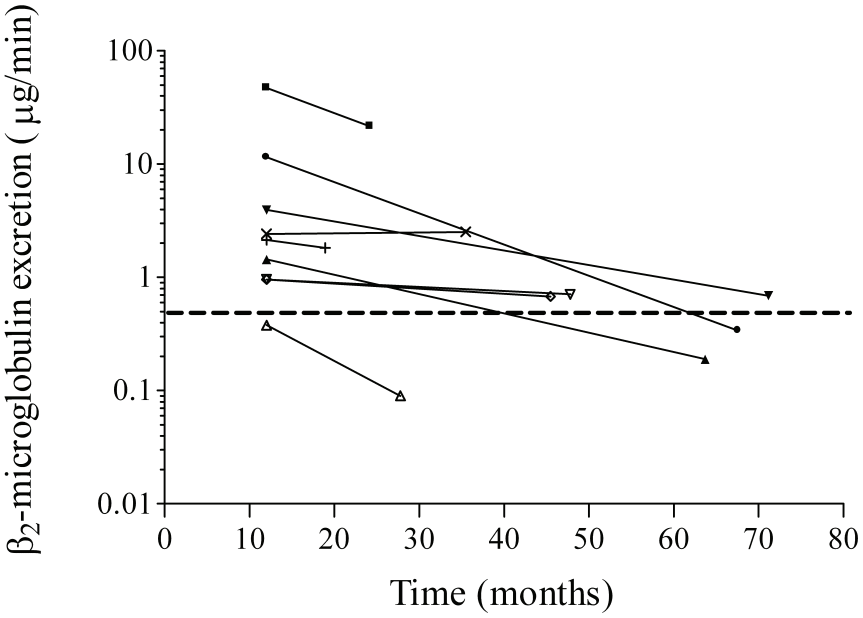


Figure 2. Progressive improvement in urinary β_2-microglobulin-excretion in patients with a stable remission.

Reference line represents threshold value.

Discussion

We previously have shown that the urinary excretion of the low molecular weight proteins β_2m and α_1m and the high molecular weight protein IgG accurately predicted renal outcome in patients with idiopathic membranous nephropathy.[5-7] In multivariate analysis urinary β_2m excretion proved 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 now evaluated the effect of this therapy on glomerular permeability and tubular proteinuria. Furthermore, we aimed at determining 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 ACE-inhibitors and/or AT1-receptor antagonists 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 attributed to these drugs since the use of these medications did not change during the treatment year. Thus, the improvement in renal function and proteinuria must be attributed to the immunosuppressive therapy.

In these 11 patients, immunosuppressive therapy resulted in a rapid improvement in glomerular permselectivity and tubulo-interstitial injury as reflected by the lower selectivity index and the decreased excretion of the low molecular weight proteins β_2m and α_1m.

Although improvement was already noted at 2 months after 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 further confirmed by the 12 month data in the group of 25 patients. Urinary β_2m was abnormal in 20 out of 21 evaluated patients, and above our previously established threshold of 0.5 μg/min in 13 patients.

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 long-term outcome after treatment. For this analysis we could not use renal insufficiency as 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 end point. Results of the analysis must be interpreted with some reluctance, since we have observed only 6 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, measurement of these various high and low molecular proteins has no value when patients have been treated.

Our study does allow drawing conclusions on some other aspects of low molecular weight proteinuria.

We have examined in more detail the possible effects of IgG on tubular reabsorption of β_2m. It is clear that the urinary IgG has only a limited effect on tubular reabsorption of β_2m, if at all.

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.

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 excretions of IgG, β_2m and α_1m. However, levels of tubular proteinuria remain significantly abnormal at the end of treatment year. Unfortunately, values at the end of the treatment year do not allow predicting long-term prognosis. The increased urinary excretion of β_2m in patients with a nephrotic syndrome is not the result of inhibition of tubular reabsorption by IgG. Thus, urinary β_2m is an accurate marker of tubular damage.

References

1. Haas M, Meehan SM, Karrison TG, Spargo BH. Changing etiologies of unexplained adult nephrotic syndrome: a comparison of renal biopsy findings from 1976-1979 and 1995-1997. *Am J Kidney Dis* 1997; 30: 621-631
2. Donadio JV, Jr, Torres VE, Velosa JA, *et al*. Idiopathic membranous nephropathy: the natural history of untreated patients. *Kidney Int* 1988; 33: 708-715
3. Schieppati A, Mosconi L, Perna A, *et al*. Prognosis of untreated patients with idiopathic membranous nephropathy. *N Engl J Med* 1993; 329: 85-89
4. Ponticelli C, Zucchelli P, Passerini P, *et al*. A 10-year follow-up of a randomized study with methylprednisolone and chlorambucil in membranous nephropathy. *Kidney Int* 1995; 48: 1600-1604
5. Reichert LJ, Koene RA, Wetzels JF. Urinary IgG excretion as a prognostic factor in idiopathic membranous nephropathy. *Clin Nephrol* 1997; 48: 79-84
6. Reichert LJ, Koene RA, Wetzels JF. Urinary excretion of β_2-microglobulin predicts renal outcome in patients with idiopathic membranous nephropathy. *J Am Soc Nephrol* 1995; 6: 1666-1669
7. Branten AJ, du Buf-Vereijken PW, Klasen IS, *et al*. Urinary excretion of β_2-microglobulin and IgG predict prognosis in idiopathic membranous nephropathy: A validation study. *J Am Soc Nephrol* 2005; 16: 169-174
8. Bazzi C, Petrini C, Rizza V, *et al*. Urinary excretion of IgG and α_1-microglobulin predicts clinical course better than extent of proteinuria in membranous nephropathy. *Am J Kidney Dis* 2001; 38: 240-248
9. Myers BD, Guasch A. Selectivity of the glomerular filtration barrier in healthy and nephrotic humans. *Am J Nephrol* 1993; 13: 311-317

10. Magil AB. Tubulointerstitial lesions in human membranous glomerulonephritis: relationship to proteinuria. *Am J Kidney Dis* 1995; 25: 375-379

11. D' Amico G, Ferrario F, Rastaldi MP. Tubulointerstitial Damage in Glomerular-Diseases - Its Role in the Progression of Renal Damage. *Am J Kidney Dis* 1995; 26: 124-132

12. D'Amico G, Bazzi C. Pathophysiology of proteinuria. *Kidney Int* 2003; 63: 809-825

13. Branten AJ, Wetzels JF. Short- and long-term efficacy of oral cyclophosphamide and steroids in patients with membranous nephropathy and renal insufficiency. *Clin Nephrol* 2001; 56: 1-9

14. du Buf-Vereijken PW, Branten AJ, Wetzels JF. Cytotoxic therapy for membranous nephropathy and renal insufficiency: improved renal survival but high relapse rate. *Nephrol Dial Transplant* 2004; 19: 1142-1148

15. Christensen CK, Mogensen CE, Hanberg SF. Renal function and cimetidine. Urinary albumin and β_2-microglobulin excretion and creatine clearance during cimetidine treatment. *Scand J Gastroenterol* 1981; 16: 129-134

16. Jacobs EM, Vervoort G, Branten AJ, *et al*. Atrial natriuretic peptide increases albuminuria in type I diabetic patients: evidence for blockade of tubular protein reabsorption. *Eur J Clin Invest* 1999; 29: 109-115

17. Branten AJ, Wetzels JF. Influence of albumin infusion on the urinary excretion of β_2-microglobulin in patients with proteinuria. *Nephron* 1999; 81: 329-333

18. Thomas DM, Hillis AN, Coles GA, Davies M, Williams JD. Enalapril can treat the proteinuria of membranous glomerulonephritis without detriment to systemic or renal hemodynamics. *Am J Kidney Dis* 1991; 18: 38-43

19. Campbell R, Sangalli F, Perticucci E, *et al*. Effects of combined ACE inhibitor and angiotensin II antagonist treatment in human chronic nephropathies. *Kidney Int* 2003; 63: 1094-1103

20. Lee TM, Su SF, Tsai CH. Effect of pravastatin on proteinuria in patients with well-controlled hypertension. *Hypertension* 2002; 40: 67-73

Chapter 9

Idiopathic Membranous Nephropathy: Outline and Rationale of a Treatment Strategy

Peggy W.G. du Buf-Vereijken[1,2], Amanda J.W. Branten[1], Jack F.M. Wetzels[1]

[1]Department of Nephrology, Radboud University Nijmegen Medical Center, Nijmegen,
[2]Department of Internal Medicine, Amphia Hospital, Breda,
The Netherlands

Am J Kidney Dis 2005; in press in revised form

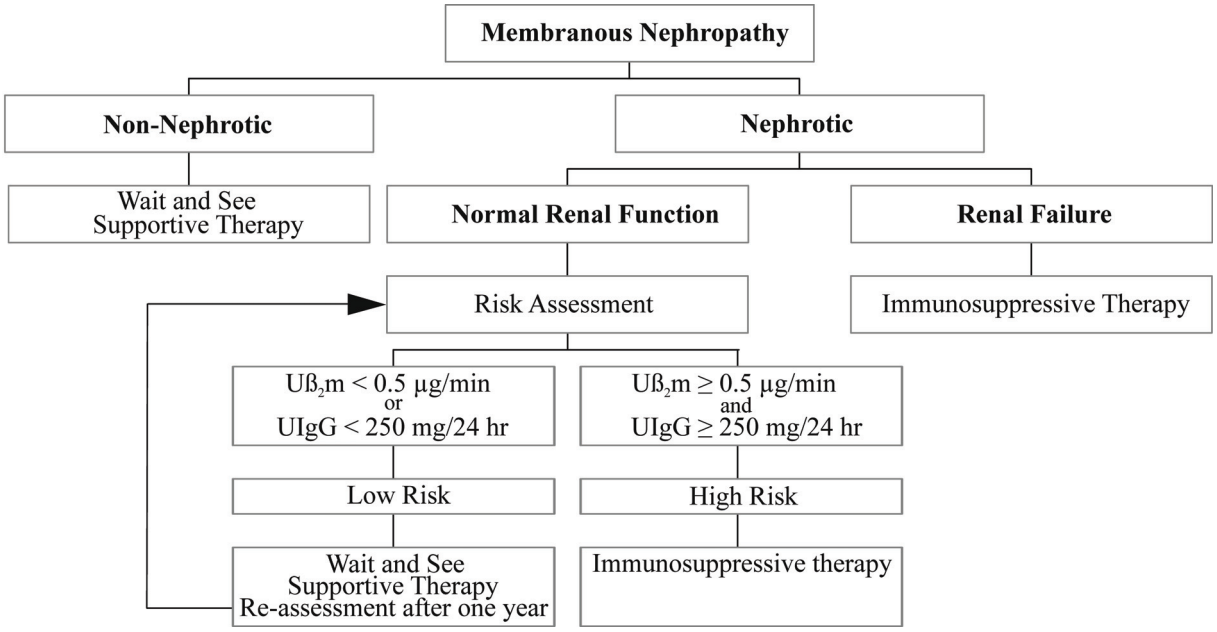
Abstract

Idiopathic membranous nephropathy (iMN) is a common cause of the nephrotic syndrome. The treatment of patients with iMN is heavily debated. Based upon literature data and our own experience we propose a rational treatment strategy. Patients with renal insufficiency are at highest risk for development of end-stage renal disease (ESRD) and should receive immunosuppressive therapy. In patients with normal renal function risk for developing ESRD can be estimated by measuring urinary excretion of β_2-microglobulin or α_1-microglobulin and IgG. For low-risk patients a wait and see policy is advised. High-risk patients likely benefit from immunosuppressive therapy. Currently, combinations of steroids with chlorambucil or cyclophosphamide are best studied. We prefer cyclophosphamide in view of the fewer side effects. Cyclosporine may be an alternative option in patients with well-preserved renal function, although long-term data are lacking. Other immunosuppressive agents such as mycophenolate mofetil or rituximab are currently under study, however there are insufficient data to support their routine use.

Introduction

Idiopathic membranous nephropathy (iMN) is one of the most common causes of the nephrotic syndrome in adult patients.[1] The natural history varies from a spontaneous complete remission of proteinuria to rapid progression to end-stage renal disease (ESRD). The treatment of iMN has been a regular theme for debate. The opinions of various investigators are as diverse as the reported data on the natural history. Some emphasize the high rate of spontaneous remissions and argue against the use of immunosuppressive drugs,[2] whereas others point to the high rate of ESRD and favour immunosuppressive therapy.[3] The titles of editorial reviews written during the past 25 years clearly reflect the uncertainty in this field, from Stewart Cameron's "Membranous nephropathy: the treatment dilemma" in 1982 and "Membranous Nephropathy-still a treatment dilemma" in 1992 to Glassock's "The treatment of idiopathic membranous nephropathy: a dilemma or a conundrum" in 2004.[4-6] In the current era of evidence based medicine some might argue that the discussion can end with the publication of a recent meta-analysis on immunosuppressive therapy for iMN.[7] Based on data derived from 18 randomized controlled trials (RCTs) including more than 1000 patients the investigators concluded that immunosuppressive treatment had no benefit in terms of patient and/or renal survival. There was weak evidence in favour of regimens containing alkylating agents in inducing a complete remission of proteinuria, however only when considering patients with relatively well preserved renal function. Since the use of immunosuppressive therapy in especially this latter group of patients is most questionable, also this finding seems to argue against the use of immunosuppressive therapy. However, the conclusions of the meta-analysis are debatable and must not lead to therapeutic nihilism. Specifically, the meta-analysis has included RCTs of limited size and quality. Conclusions based on a systematic review, which includes many trials of limited quality are not necessarily better than conclusions based on the results of one large, carefully conducted randomized controlled trial. Furthermore, in view of the limited number of large, high-quality RCTs, we must not neglect important and relevant information that can be obtained from carefully conducted observational studies.[8;9]

During the past two decades we have systematically studied patients with iMN, our database now includes 279 patients.[8;10-18] These studies have enabled us to define risk factors and to develop a treatment strategy tailored to the individual patient. Our treatment strategy is depicted in Figure 1.



Schedule Immunosuppressive Therapy:
Methylprednisolone 1 g i.v. days 1,2,3, 61,62,63,121,122,123
Prednisone 0.5 mg/kg every other day 6 months
Cyclophosphamide 1.5 mg/kg/day 12 months

Figure 1. Outline of proposed treatment strategy in patients with idiopathic membranous nephropathy.

In this review we discuss treatment modalities for patients with iMN and provide compelling arguments based on literature data and our experience in favour of our strategy. We specifically address the following questions.

1. Has the natural history of iMN changed during the past decades?
2. Is immunosuppressive therapy of proven benefit in patients with iMN when considering hard end-points?
3. Should all patients with iMN and nephrotic syndrome be treated with immunosuppressive therapy?
4. Are all immunosuppressive agents equally effective?
5. Which parameters can be used to identify patients at risk for progressive renal insufficiency?

1. The natural history of idiopathic membranous nephropathy.

It is important to define the natural history of iMN. In fact, most will probably agree that the overall prognosis determines if one would ever consider the (early) use of aggressive therapy. In this respect, descriptions of the natural history of iMN are quite divergent and thus have laid the ground for heavy disputes on the use of immunosuppressive therapy. Schieppati *et al.* have pointed to the relatively benign course of iMN in untreated patients, with 65% of patients who were followed for more than 5 years developing a spontaneous remission of proteinuria and an estimated renal survival rate of 88% at 5 years.[2] In contrast, Ponticelli *et al.* have stressed the poor outcome observing a permanent remission in only 33% of untreated patients followed for more than 10 years and a renal survival rate of 60% at 10 years.[3] It is no surprise that the first investigators claim that symptomatic treatment is still the best option for patients with iMN whereas the latter argue that all patients with iMN and a nephrotic syndrome should receive immunosuppressive therapy.

The short-term outcome (< five years) of membranous nephropathy already was reported extensively before 1980.[19-26] However, it is difficult to compare results because most of these older studies have the handicap that they included not only patients with idiopathic and secondary membranous nephropathy, but also treated and untreated patients. In the above-mentioned studies a complete remission of proteinuria occurred in about 16% to 29% of patients, whereas in about 40 – 60% of patients, there was evidence of progressive renal insufficiency.

To better appreciate the natural history of patients with iMN we have analysed studies published during the past 25 years. An overview is given in Tables 1 and 2.[2;3;27-36] Whenever possible we have used the data from untreated patients. The reported studies still vary considerably with respect to patient characteristics, time of follow-up and definition of renal failure. Therefore, it may be no surprise that reported outcomes are quite variable. However, we must take into account the fact that many studies have included patients with non-nephrotic proteinuria. Outcome in never-nephrotic patients with iMN is invariably good, with reported 10-year renal survival rates approximating 100%.[31;35;37-39]

Table 1. Natural history of patients with idiopathic membranous nephropathy

Author	Yr	Patients (*n*)	Sex M/F	Screat (mg/dl)*	Proteinuria (g/day)	NS (%)	Treated (%)	FU (Yr)	CR (%)	PR (%)	RFD (%)	ESRD (%)
Coggins[27]	1979	38	20/18	1.0±0.2	8.3±4	100	0	2	13	16	29	21
Davison[28]	1984	64	47/17	1.24	NA	81	0	2-15	NA	NA	50	??
MacTier[29]	1986	37	31/6	1.47	10.5	93	0	5.3	22	8	41	22
Zuchelli[30]	1987	49	37/12	1.18±0.27	NA	100	0	9.5	14.3	40	45	27
Donadio[31]	1988	89	56/33	1.3±0.6	8.2±5.2	83	0	6.1	NA	NA	33	18
Cattran[32]	1989	77	44/33	1.17±0.1	5.2±0.9	73	0	4	35	23[#]	25	5
Wehrmann[33]	1989	334	223/111	74/334 Screat>1.3	NA	73	35	4	NA	NA	31	14
Cameron[34]	1990	51	43/8	1.3±0.49	10.4±5.3	100	0	4.3		14	52	29
Durin[35]	1990	82	48/34	9/82 Screat>1.5	NA	68	0	8.5		39	38	20
Schieppati[2]	1993	100	68/32	1.1±0.5	5.1±3.6	63	0	3.3	22	44	32	14
Ponticelli[3]	1995	39	29/10	1.05±0.29	5.3±2.8	100	0	>10	5	28	47	25

Screat, serum creatinine. *To convert serum creatinine in mg/dl to µmol/l multiply by 88.4.
NS, nephrotic syndrome; FU, duration of follow-up; CR, complete remission; PR, partial remission;
RFD, renal function deterioration; ESRD, end-stage renal disease; NA, not available. [#]Remission at 3 years of follow-up.
Durin[35] provides long-term outcome of a patient cohort originally described by Noel in 1979.[36]

The confounding effect of including up to 37% of non-nephrotic patients in studies of the natural history of iMN is illustrated in Figure 2.

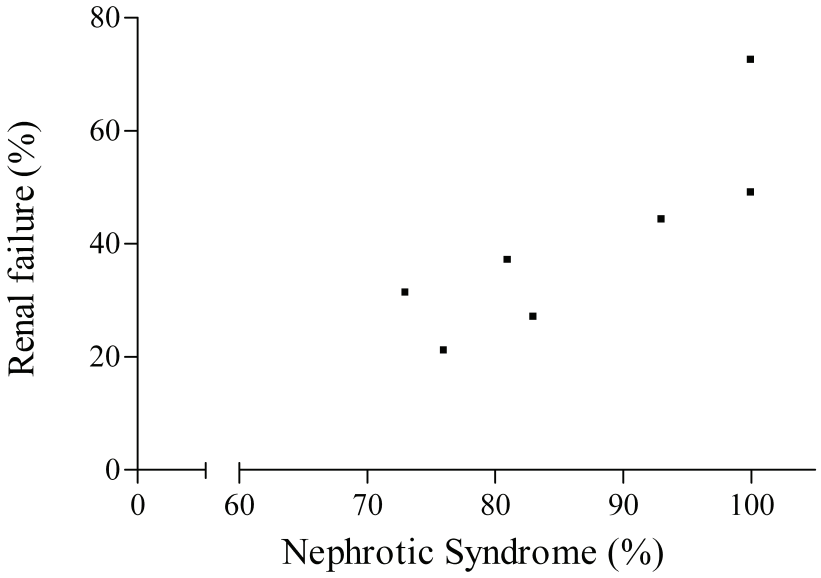


Figure 2. Outcome in studies of patients with idiopathic membranous nephropathy is dependent on the prevalence of the nephrotic syndrome.

Relation of the calculated percentage of patients with progressive renal insufficiency during 5 yr follow-up and the percentage of patients with a nephrotic syndrome in studies on membranous nephropathy. Data from references.[27-29;31;32;36;61]

Therefore, we have recalculated the data of the studies in Table 1 by attributing a 100% renal survival rate to the non-nephrotic patients (Table 2). Furthermore, we have not only included the reported data on the percentage of patients with ESRD, since these figures are not always corrected for patient death and not quite informative for studies with follow-up of less than 5 years. To circumvent this problem and to allow good comparisons between studies we have calculated the percentage of patients with evidence of renal function deterioration (RFD), which is a very specific predictor of ESRD.[37] From Table 2 it is evident that the data become more homogeneous. Overall, nearly half of the patients with iMN and a nephrotic syndrome will develop renal failure. The validity of our assumptions is underlined by the good agreement between the calculated percentage of RFD and the reported overall renal survival rate (Table 2).

Table 2. Calculated outcome in patients with idiopathic membranous nephropathy and a nephrotic syndrome

Author	Yr	Patients (n)	Nephrotic (%)	Treated (%)	Follow-up (Yr)	Corrected RFD (%)	Corrected renal survival (%) (Yrs)
Davison[28]	1984	64	81	0	7	62	NA
MacTier[29]	1986	37	93	0	5.3	44	46
Zuchelli[30]	1987	49	100	0	9.5	45	52 (10 yr)
Donadio[31]	1988	89	83	0	6.1	39	49 (10 yr)
Cattran[32]	1989	77	73	0	4	34	88 (8 yr)*
Wehrmann[33]	1989	334	73	35	4	42	59 (4 yr)
Cameron[34]	1990	51	100	0	4.3	52	NA
Durin[35]	1990	82	68	0	8	56	63 (10 yr)
Schieppati[2]	1993	100	63	0	3.3	51	57 (8 yr)
Ponticelli[3]	1995	39	100	0	> 10	47	60 (10 yr)

RFD, renal function deterioration; we calculated percentage RFD and renal survival after correction for the percentage of patients without a nephrotic syndrome assuming a 100% survival in non-nephrotics. The correction factor used = 100/(% Nephrotics).
For this analysis we excluded studies with a follow-up < 3 yrs.[27] NA, not available.
* The projected eight years renal survival is not reliable, since 22% of patients were lost to follow-up and median follow-up time was only 4 years.

Obviously, conservative treatment of patients with proteinuria has changed dramatically in the past decade. Nowadays all patients with proteinuria are treated with ACE-inhibitors (ACEi) or angiotensin II type 1 receptor antagonists (ARBs). These agents reduce proteinuria and attenuate the deterioration in renal function in patients with diabetic and non-diabetic proteinuric renal diseases.[40-43] Therefore, one might question the relevance of the data presented in Tables 1 and 2, which are largely derived from studies that included many patients who did not receive ACEi or ARBs. We must consider if the natural history of iMN has changed with the venue of ACEi and ARBs.

Indeed, the prospect of the use of ACEi as effective treatment in patients with iMN has stimulated the initiative of a randomized study in the early nineties. In this study, called ACIMEN (ACE inhibition versus Corticosteroids in Membranous Nephropathy) it was intended to compare the ACEi enalapril with a 6-month course of alternate day prednisone or supportive treatment.[44] Unfortunately, this study has not been completed due to the low rate of patient accrual; however, an interim analysis did not reveal any particular benefit of ACEi over placebo.

To determine whether use of ACEi could have substantially changed the prognosis in patients with iMN we have analysed the outcome in patients with iMN entered in our database since 1988. For this analysis we have included all patients with a biopsy proven idiopathic MN, a normal renal function (serum creatinine < 1.25 mg/dl*) at the time of biopsy, and treated with an ACEi or an ARB (start of treatment before or within 6 months after biopsy). There were 91 patients (61M, 30F) who fulfilled the entry criteria. Median age was 49 years (range 18-78), serum creatinine 0.98 mg/dl (0.54 – 1.24), and proteinuria 6.1 g/10 mmol creatinine (0.7 – 32). A nephrotic syndrome was present in 87% of patients. Median follow-up was 46 months (3-167). During follow-up 39 patients (43%) have developed renal death, defined by the criteria that we have regularly used to allow the start of immunosuppressive therapy.[8;15] Thus, our data indicate that the use of ACEi or ARBs has not greatly improved the prognosis in patients with iMN. Our data support the findings of Troyanov *et al.*, who assessed the role of ACEi as independent predictor of outcome in their cohort of patients with iMN.[45] In multivariate analysis the use of ACEi was not related to outcome. The possible benefits of ACEi treatment in patients with iMN have also been challenged by the studies of Praga *et al.*, who clearly demonstrated that the antiproteinuric effects of ACEi were particularly evident in patients with renal diseases characterized by secondary focal segmental glomerulosclerosis due to hyperfiltration.[46] In these patients proteinuria decreased from 7.1 ± 1.7 g/day at baseline to 3.7 ± 1.7 g/day after 6 months of treatment with ACEi. In contrast, in patients with a nephrotic syndrome (the majority caused by iMN) proteinuria remained unchanged at approximately 8 g/day. In a subsequent study it was shown that this poorer antiproteinuric response in patients with primary glomerulopathies also heralded a worse outcome with respect to renal function.[47]

From the studies that report on the natural history important information can be obtained on the time course of events in patients with iMN. This knowledge is pivotal to allow evaluation of the quality of RCTs conducted in patients with iMN, in particular, to determine whether suitable end-points have been used in relation to the time of follow-up. In general, development of ESRD will take more than 5 years, and as a consequence studies that use ESRD as end point need a follow-up of 7-10 years. In contrast, patients with evidence of RFD (a specific predictor of ESRD, see below) can be identified at an earlier time point. In various studies the median time to the development of renal insufficiency was 2-2.5 years, with no patient with a normal renal function at 5 year follow-up showing deterioration of renal

function thereafter.[28;35] Thus, RFD can be used as an estimate of treatment efficacy in studies with a follow-up of 3-4 years.

The remission rate cannot be evaluated at a much earlier time point. The median time to partial remission ranges from 11 to 23 months and to complete remission from 16 to 40 months.[8;38;45;48] Although remissions occurred somewhat earlier in treated patients, the median time to complete remission in Ponticelli's and our studies was 18 and 22 months respectively.[3;8] Thus, studies with a limited time of follow-up (less than 2-3 years) cannot be used to evaluate remission rate.

Admittedly, it can be questioned whether RFD and remission rate can be used as reliable surrogate end points of studies in iMN. The use of RFD as end point is supported by studies showing low renal survival in untreated iMN patients with established renal insufficiency.[8;9] Furthermore, patients with evidence of RFD (an increase of serum creatinine) almost invariable progress to ESRD.[28;37;49] Likewise, the development of a remission can be used as a surrogate end point of a study, because most studies have documented a good overall prognosis in patients who have entered a partial or complete remission of proteinuria, independent of treatment.[38;45;48;50] In Troyanov's study the hazard ratio for developing ESRD was zero for patients with a complete remission and 0.08 for patients with a partial remission.[45]

2. Is immunosuppressive therapy of proven benefit?

In the period 1960-1970 membranous nephropathy was considered a slowly progressive disease, that was totally unresponsive to steroid treatment (reviewed by Rastogi *et al.*).[51] However, several investigators thereafter have reported complete remissions of proteinuria in patients with iMN following steroid therapy. Rastogi *et al.* have summarized the data of 108 patients who were treated with steroids, 29 patients developing a complete remission of proteinuria and 19 patients developing a partial remission. Since many of the treated patients had a normal renal function at the time of treatment start, these data didn't provide hard evidence that treatment with prednisone improved outcome.

A subsequent randomized controlled trial provided promising results.[27] A treatment regimen consisting of high dose alternate day prednisone (125 mg every other day for 8 weeks) significantly reduced the rate of renal function deterioration. This study has been criticized because of the rather high rate of doubling of serum creatinine that occurred within two years of follow-up in the placebo group (29% *vs* 6%). Two subsequent RCTs unequivocally proved

that prednisone did not prevent deterioration of renal function.[32;34] Apparently publication of these RCTs has settled the issue, and prednisone monotherapy since then is regarded ineffective in patients with iMN. However, it is important to realize that these conclusions only hold for the use of prednisone in limited dosage or during a limited time period. The above-mentioned studies have used either 125-150 mg prednisone on alternate days for 8 weeks[27;34] or 45 mg/m^2 on alternate days for 6 months,[32] thus providing cumulative doses of prednisone of 4.2 and 7.0 g respectively. It is quite possible that a higher dose of prednisone administered for a longer period of time may be more effective. Hopper *et al.* have used prednisone in a dose of 100-200 mg every other day for a period of 7.5 months, followed by a gradual dose reduction during another 6 months.[52] The cumulative dose of prednisone averaged > 25 g. They have reported on 15 patients with progressive disease during an observation period of 8-66 months before start of therapy. After treatment 7 patients have developed a complete remission and 4 a partial remission of proteinuria. Before start of therapy renal function was severely reduced in 9 patients (all with serum creatinine > 1.8 mg/dl). At the end of follow-up renal function had improved in 7 out of these 9 patients. Slightly less impressive results were reported by Short *et al*.[53] These investigators have used pulses methylprednisolone (5x 1000 mg), followed by high dose oral prednisone in a starting dose of 100 mg every other day, with gradual reduction over 8 months (cumulative dose approximately 9.5 g). Fifteen patients with renal insufficiency were treated and an improvement in renal function was noted in all, mean serum creatinine decreasing from 4.6 to 2.5 mg/dl, the average percentage reduction amounting 46% (21% to 65%). Although improvement was not sustained in all, still 8 patients were alive without renal failure after 6-48 months follow-up. Other uncontrolled studies that have reported some benefit from steroid therapy have used prednisone in cumulative dosages of 9-10 g.[20;54] Admittedly, the high dose prednisone regimen as used by Hopper is quite toxic and its efficacy is not adequately proven. Therefore, it is realistic to consider alternative treatment options.

Other immunosuppressive agents have been used in patients with iMN, although most regimens have contained prednisone. Most studies have used a combination of an alkylating agent and prednisone.[3;8;9;14;15;55-64] The efficacy of combined immunosuppressive therapy in membranous nephropathy was illustrated in an interesting case report by Ford.[65] The author described a patient with ESRD due to iMN who received a kidney transplant. Postoperatively a renal biopsy was done because of the slower than expected improvement in renal function. The biopsy contained only totally infarcted renal tissue, which was confirmed by transplant

nephrectomy. Renal function meanwhile had gradually improved to a creatinine clearance of 25 ml/min, with proteinuria averaging 1.6 g/day. This patient needed no further dialysis during 2 years of follow-up.

The best study on the efficacy of aggressive immunosuppressive therapy in patients with iMN undoubtedly is the randomized controlled trial conducted by Ponticelli and co-workers.[3;61;62] These Italian investigators have randomized patients with iMN with a nephrotic syndrome and normal renal function for treatment with alternating monthly cycles of prednisone and chlorambucil versus no treatment. Duration of treatment was 6 months. Patients have meanwhile been followed for more than 10 years.[3] The data unambiguously have demonstrated the beneficial effect of immunosuppressive therapy. Treatment increased remission rate (at the end of follow-up 63% *vs* 33%) and improved renal survival (92% *vs* 60%).

Unfortunately, the results of one RCT cannot be used to draw conclusions with the highest level of evidence. In the recently published Cochrane meta-analysis the results provided by Ponticelli's study are virtually annihilated by reports of three other RCTs. However, two RCTs were small sized and had a limited follow-up of 12 and 24 months respectively.[66;67] In view of the above-mentioned characteristics of the natural course in iMN, these latter studies cannot be used to analyse the effect of treatment on renal function. Notably, even within the short period of follow-up both studies documented a significant lower proteinuria in the treated patients. The third RCT has only been published in abstract form.[68] However, additional information is provided in the manuscript by Risler *et al*.[69] The investigators specifically state that for statistical purposes retrospectively studied control patients were added, thus invalidating this study as RCT. Furthermore, no clinical data are provided, especially the percentage of nephrotic patients is unnoted. Moreover, chlorambucil and prednisone tended to be more effective in inducing remission of proteinuria in the severely nephrotic patients and were of limited effect in the absence of tubulo-interstitial lesions.

The efficacy of alkylating agents in patients with iMN is supported by other studies. In a small RCT we have demonstrated that the Ponticelli regimen is more effective than i.v. cyclophosphamide and methylprednisolone.[10] Furthermore, two cohort studies have demonstrated the efficacy of alkylating agents.[8;9] In these studies historical controls were used for comparison. The data of these studies are strengthened by the fact that only patients with RFD were included, thus patients with a bad renal prognosis and a low likelihood of spontaneous remissions. Torres *et al.* have treated patients with iMN and renal insufficiency

with chlorambucil and prednisone. We have performed a similar study using a cyclophosphamide-based regimen. Results were quite similar, with a favourable renal survival in the treated patients when compared with the historical controls (Figure 3).

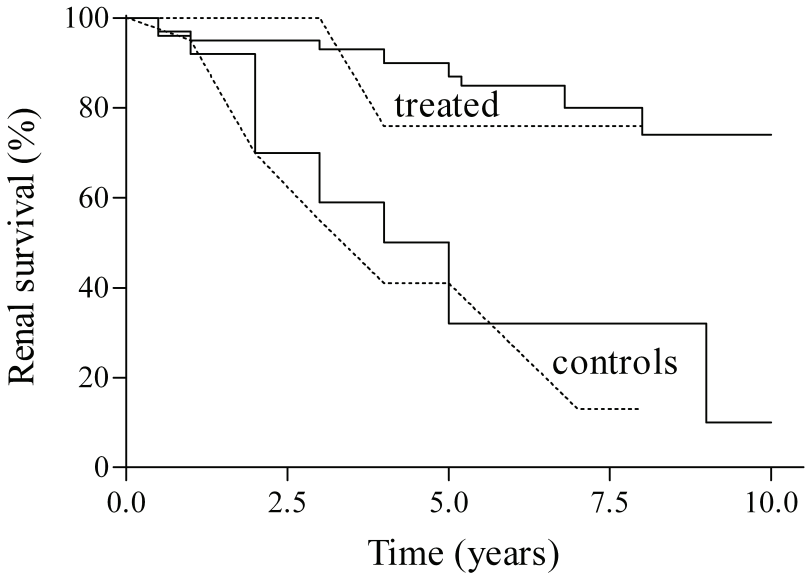


Figure 3. Renal survival in patients with idiopathic membranous nephropathy and renal insufficiency treated with alkylating agents (treated) compared with historical controls (controls).

Data adapted from Du Buf *et al.*[8] (cyclophosphamide; straight lines) and Torres *et al.*[9] (chlorambucil; dotted lines). Data of Torres *et al.* were recalculated to provide overall renal survival without censoring for death.

In conclusion, a high-quality RCT and two cohort studies with historical controls, of adequate size and long follow-up provide evidence for the efficacy of immunosuppressive therapy consisting of alkylating agents and prednisone in patients with iMN.

3. Should all patients with membranous nephropathy and a nephrotic syndrome be treated?

Based on the results from their controlled trials Ponticelli *et al.* concluded that treatment with chlorambucil and prednisone improved survival in patients with iMN, a nephrotic syndrome and normal renal function.[61] Although we fully appreciated their findings, we and others were not convinced that the data proved that all patients should receive immediate treatment. Adoption of such an approach would unnecessarily expose up to 40% of patients to toxic immunosuppressive agents.

From the late 1980s, we have adopted a restricted treatment policy in which immunosuppressive therapy was given to patients with iMN, a nephrotic syndrome, and evidence of renal function deterioration.[13;14;70] We found support for this strategy in studies by Mathieson and Warwick who reported improvement in renal function in patients with renal insufficiency.[60;64] We recently have reported our experience with a cyclophosphamide based treatment regimen in 65 patients with iMN and renal insufficiency.[8] Renal function improved at least temporarily in > 90% of patients, and the cumulative incidence of complete and partial remissions of proteinuria was 92% at 5 years. If we calculated renal survival from the time of biopsy renal survival rates were 93% and 81% at 5 and 10 years respectively. Although these results were quite favourable and supported the efficacy of immunosuppressive therapy when started in patients with renal insufficiency, these data could not answer the question if start of therapy can be safely delayed until renal insufficiency has developed. In fact, our survival data must be compared with the results obtained by Ponticelli *et al.* who reported a 10 years renal survival of 92% (Figure 4).

It is obvious that the comparison is biased in view of the high-risk profile of our treated patients. Therefore we have formally analysed the results of our restrictive treatment policy in an unbiased cohort of patients with iMN, a nephrotic syndrome and normal renal function at the time of biopsy.[16] We advised to restrict immunosuppressive treatment to patients with renal insufficiency. The cohort comprised 69 patients with a serum creatinine of 1.0 ± 0.2 mg/dl and proteinuria 6.7 ± 3.0 g/day. Follow-up was 5.4 (0.5-14.1) years. Thus far 33 patients (48%) have received immunosuppressive therapy, which confirms the general idea that approximately half the patients will not need therapy.

If we calculated renal survival for the patients who were treated according to the protocol 5-year renal survival was 97%, comparable to the results obtained by Ponticelli *et al.* when treating all patients from the onset of disease (Figure 4). There was a small survival difference at 10 years of follow-up. However, it is important to realize that the average serum creatinine in our treated patients was 1.7 ± 0.6 mg/dl at the time of start of therapy whereas in Ponticelli's study serum creatinine was 1.05 ± 0.25 mg/dl. It is likely that our results had been even better if treatment in the high-risk patients had started at an earlier time point, which may become possible with the use of sensitive and specific predictors of progression (vide infra).

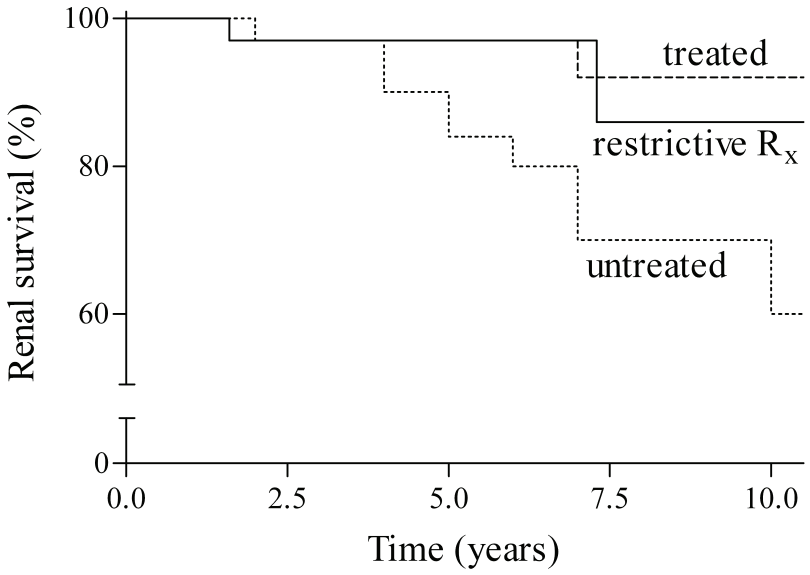


Figure 4. Renal survival in patients with iMN, a nephrotic syndrome and normal renal function using a restrictive treatment policy.[16]

Overall, 48% of patients have needed immunosuppressive treatment during follow-up. For comparison renal survival is shown for patients included in the RCT of Ponticelli *et al.* comparing chlorambucil and steroids (treated) with no treatment (untreated).[3]

4. Are all immunosuppressive agents equally effective?

Various immunosuppressive agents have been used in the treatment of patients with iMN including chlorambucil, cyclophosphamide, azathioprine, cyclosporine, mycophenolate mofetil, tacrolimus, ACTH, and most recently the anti CD20 monoclonal antibody rituximab and the anti-C5a monoclonal antibody eculizumab.[3;8;9;14;15;55-60;62;63;66;67;69;71-88] Relevant data from the most important studies are given in Tables 3 and 4.

There are no randomized trials that have compared the various classes of agents. It therefore is difficult to draw hard conclusions. From reviewing the literature some conclusions emerge.

Most studies have used oral chlorambucil or oral cyclophosphamide. Our experience with both agents has been reported previously.[14] In our hands, a regimen based on chlorambucil was less effective and more toxic than a cyclophosphamide based regimen. An overview of the studies performed in patients with renal insufficiency supported this notion (Table 3).

Table 3. Results of treatment with cyclophosphamide or chlorambucil in patients with idiopathic membranous nephropathy.

Reference	Patients (*n*)	Sex (M/F)	Screat (mg/dl)	Prot (g/day)	Follow-up (months)	Remission Proteinuria Initial CR	Initial PR	Final CR	Final PR	Renal Function IM	S	D
Cyclophosphamide												
Bruns[56]	11	9/2	2.24 (1.80-4.20)	11.9 (6.2-22)	34 (12-54)	4	5	3	4	7	4	0
Jindal[59]	9	7/2	2.51 (1.24-3.74)	11.1±7.6	83±13	4	4	3	4	3	3	3
Alexopoulos[72]	17	12/5	1.30±0.5	5.1±1.4	58.9 (12-156)	5	7	3	4	1	12	4
Faedda[57]	4	NA	>2.26	NA	144	3	1	3	1	3	1	0
Du Buf[8]	65	55/10	1.93 (1.20-5.79)	10 (2-23)	51(5-132)	17	39	16	31	35	20	10
Total	106					33 (31%)	56 (53%)	28 (26%)	44 (42%)	49 (46%)	40 (38%)	17 (16%)
Chlorambucil												
Mathieson[60]	8	7/1	2.19±0.7	15.3 (8.8-23.9)	17 (9-32)	1	3	0	4	6	1	1
Brunkhorst[55]	9	6/3	2.57±0.44	>10	20 (12-24)	NA	NA	NA	NA	5	2	2
Warwick[63]	21	19/2	2.71 (2.04-5.43)	14.1 (1.4-30.4)	39 (4-68)	NA	NA	2	4	7	5	9
Stirling[83]	19	17/2	3.02	12.5	54	NA	NA	2	3	5	2	12
Branten[14]	15	15/0	2.48±0.83	9±2.6	38 (8-71)	0	5	0	2	3	3	9
Torres[9]	19	11/8	2.3±0.94	11.2±3.3	51.8±36.5	5	3	5	2	11	2	6
Total	91							9# (11%)	15# (18%)	37 (41%)	15 (16%)	39 (43%)
Cyclophosphamide												
Ponticelli[89]	45 (43)*	29/16	1.04±0.27	6.85±3.51	42 (12-72)	16	24	16	24	0	41	2
Chlorambucil												
Ponticelli[89]	50 (44)*	37/13	1.06±0.27	7.96±5.19	36 (12-78)	12	24	12	24	0	43	1

Screat, serum creatinine; prot, proteinuria; CR, complete remission; PR, partial remission; IM, improved; S, stable; D, deteriorated; NA, not available;
* number of patients at final assessment is given in brackets; # percentage of patients in remission calculated for $n = 82$ evaluated patients.

Table 4. Results of treatment with azathioprine, cyclosporine or MMF in patients with idiopathic membranous nephropathy.

Reference	Patients (*n*)	Sex (M/F)	Screat (mg/dl)	Prot (g/day)	Follow-up (months)	Remission Proteinuria Initial CR	Initial PR	Final CR	Final PR	Renal Function IM	S	D
Azathioprine												
Baker[74]	6	NA	(2.83-5.09)	(6-17)	12	0	0	0	0	5	1	0
Bone[73]	21	15/6	3.0 ± 0.19	12.2 ± 1.4	120 (36-240)	8	6	6	12	14	2	5
Brown[75]	13	10/3	2.38 (1.89 - 3.96)	11.8 (4.3-21.7)	73 (24-103)		6		7	11	1	1
Ahuja[71]	38	32/6	1.6 (0.7-4.9)	6.1 (3.3-20.4)	48	10	11	10	11	0	24	14
Total	78					18# (28%)	17# (26%)	16# (25%)	23# (35%)	30 (38%)	28 (36%)	20 (26%)
Cyclosporine												
Cattran[76]	9	8/1	2.1 ± 0.74	11.5 (9-18)	26 (3-36)	0	0	0	0	1	4	4
MMF												
Miller[80]	16	11/5	2.1 ± 1.3	9.2 ± 4.2	8	0	2	0	2	2	13	1
Choi[78]	17	10/7	1.5 ± 0.8	7.8 ± 4.8	12 ± 7	2	5	3	4	3	14	0
CsA (RCT)												
Cattran[77]												
CsA	28	26/2	1.3 ± 0.5	9.7 ± 5.3	17	2	19	2	9	0	26	2
Placebo	23	16/7	1.1 ± 0.3	8.8 ± 4.7	17	1	4	1	2	0	21	2

Data are provided for studies that have included high-risk patients (patients with renal failure) or for randomized controlled trails.
Screat, serum creatinine; prot, proteinuria; CR, complete remission; PR, partial remission; IM, improved; S, stable; D, deteriorated;
NA, not available; MMF, mycophenolate mofetil; CsA, cyclosporine; RCT, randomized controlled trial;
percentage of patients in remission calculated for $n = 65$ evaluated patients.

Remission of proteinuria as well as an improvement in renal function was more frequently seen during cyclophosphamide treatment. Admittedly, we cannot exclude that the better efficacy of cyclophosphamide is explained by the fact that cyclophosphamide therapy usually is given for a more prolonged period of time (12 months cyclophosphamide as compared with 6 months chlorambucil). However, despite this shorter treatment period side effects occurred more frequently with chlorambucil. It has been suggested that side effects of chlorambucil might be particular prominent in patients with renal insufficiency. However, a similar difference in side effects was noted by Ponticelli *et al.* who compared chlorambucil and cyclophosphamide, administered in monthly alternating cycles with prednisone, for 6 months in patients with normal renal function.[89] Side effects occurred more frequently during chlorambucil: *Herpes Zoster* infections occurred in 8% of chlorambucil-treated patients and in 0% of the cyclophosphamide-treated patients, other side effects necessitated withdrawal of therapy in 12% of patients on chlorambucil *vs* 4% on cyclophosphamide.
Of note, the above-mentioned studies all have used orally administered cyclophosphamide. Thus far, treatment schedules that have used intravenous pulses of cyclophosphamide have been ineffective.[10;58]

Azathioprine often is considered a good replacement of cyclophosphamide, and recent studies in patients with vasculitis have provided evidence that after the induction phase (3 months) cyclophosphamide can be replaced safely by azathioprine.[90]
We are used to switch from cyclophosphamide to azathioprine after three months in patients of young age because of the infertility risks associated with the use of cyclophosphamide.[91] Although our experience is limited we have the impression that the few treatment failures that we have observed during the past 10 years were confined mainly to patients who had used cyclophosphamide for only three months. In the literature azathioprine has been used with variable success.[71;73-75;84;85] The first study by the Western Canadian Glomerulonephritis Study Group was a controlled double blind randomized trial that included only 9 patients (5 azathioprine, 4 placebo) followed for merely one year.[84] Not surprisingly there was no proven benefit of azathioprine. A similar conclusion was reached in a retrospective study[71] that compared 38 treated patients with 20 contemporary untreated patients. Unfortunately, treatment was left at the discretion of the physicians, which makes it likely that the baseline characteristics have been pivotal in making a treatment decision. Indeed, although not significantly different, serum creatinine and proteinuria were 17 – 23% higher in the treated

patients. Furthermore, the study has included many patients with a good prognosis as reflected by the high spontaneous remission rate of 65% in the untreated patients. In a population with a good outcome in the untreated patients, it is more difficult to prove the efficacy of treatment especially if only small numbers of patients are included.

Other studies have focussed on patients with progressive renal failure and reported more promising results (Table 4).[73-75;85]

Bone *et al.* have evaluated the outcome after start of azathioprine therapy in 21 patients with evidence of progressive renal insufficiency as reflected by a mean decrease in ECC of 23 ml/min/yr.[73] These patients have been followed for 10 yrs. Treatment resulted in an improvement or stabilisation of renal function in all but three patients and a reduction of proteinuria, although levels below 1 g/day were reached in only 6 patients. At the end of follow-up 4 patients had progressed to dialysis, still 14 were alive with functioning kidneys. Of note, treatment with low dose azathioprine and prednisone needed to be continued lifelong, with relapses occurring during reductions of the prednisone dose. Roughly similar results have been reported by Brown *et al*.[75] These investigators have treated 13 patients with azathioprine and prednisone. All patients had evidence of renal failure (see Table 4). Overall treatment improved renal function with a decrease in serum creatinine > 15% in 10 patients and resulted in a complete or partial remission in 7 patients. Continued treatment was needed to maintain efficacy during follow-up. Only 4 patients were able to successfully discontinue azathioprine and prednisone therapy at the end of follow-up. The latter studies at least suggest that a combination of azathioprine and prednisone exerts beneficial effects in patients with iMN and renal insufficiency. However, the data also indicate that azathioprine containing regimens may be effective only if treatment is continued for life, in contrast to the experience with cyclophosphamide.

Cyclosporine was used with success in patients with minimal change disease. The efficacy of cyclosporine (CsA) was attributed to the ability of CsA to decrease the production of lymphokines or cytokines. Subsequently, it was noted that CsA also reduced proteinuria in patients with such non-immunological glomerular diseases as Alports syndrome.[92] In these latter patients the reduction of proteinuria was considered the consequence of the hemodynamic effects of CsA, which decreased GFR. Animal studies clearly proved a direct effect of CsA on glomerular permeability,[93;94] which was confirmed in humans. Zietse *et al.* studied patients with iMN and observed a decrease in proteinuria within 1-3 months. Because

the fractional excretion of albumin decreased, it was suggested that hemodynamic effects were not the only cause of the decrease in proteinuria. In subsequent studies, both Zietse and Ambalavanan, by using dextran sieving experiments confirmed that CsA improved glomerular permselectivity, with a reduction in shunt flow.[95;96] Unfortunately, proteinuria returned to baseline values within 4-8 weeks after stopping CsA therapy.

Many studies have demonstrated the short-term antiproteinuric effect of CsA in patients with iMN. Most investigators agree that the effect is evident within 3 months after start of therapy, and that continued use of CsA beyond 4 months is not useful in non-responders. It remained unclear if use of CsA could have long-term benefits. Rostoker *et al.* treated patients for a median of 15 months and observed that several patients remained in remission after discontinuation of the drug.[82] Obviously, in this study it was not excluded that most sustained remissions could have occurred spontaneously. Most importantly, the data did not prove or even suggest that CsA would benefit patients with iMN in terms of attenuating development of renal failure.

Cattran *et al.* have reported the results of a small RCT (including 18 patients total) with iMN and renal insufficiency.[76] They observed that CsA attenuated RFD compared with placebo, with a decrease in the slope of ECC from 2.1 to 0.7 ml/min/month. Remarkably, treatment with CsA did not result in an improvement in renal function, and no patient developed a complete remission of proteinuria, in contrast to the observed effects of alkylating agents. To date, these data have not been confirmed. In fact, others have noted that CsA was not very effective in patients with renal insufficiency, and even often caused progression to ESRD.[79] Furthermore, the Cyclosporine in Membranous Nephropathy Study group (Cyclomen) compared CsA with conservative treatment in patients with iMN and RFD.[97] In this controlled trial which was terminated too early, CsA failed to exert long term benefits. This and other observations have led Ponticelli to advise against the use of CsA in patients with a creatinine clearance < 60 ml/min and/or severe hypertension and/or severe tubulo-interstitial fibrosis and tubular atrophy at renal biopsy.[98]

The situation may be different in patients without renal failure. The efficacy of CsA was studied in an RCT of patients with iMN and normal renal function.[77] Eligible patients were steroid resistant as defined by non-responsiveness to 8 weeks prednisone. Obviously, this definition can be questioned because prednisone is not considered effective therapy and a period of 8 weeks is too short to document remissions in patients with iMN. Nonetheless, CsA significantly decreased proteinuria compared with placebo. At the end of the 26-week

treatment period of 28 CsA-treated patients, 2 patients were in complete remission and 19 patients were in partial remission, significantly different from results in the 23 untreated patients (complete remission in 1 and partial remission in 4 patients). However, after ending treatment many relapses occurred, and at the end of follow-up the differences in remission rate were not very impressive (Table 4). The follow-up of this study was too short, only 18 months, to allow conclusions with respect to renal function. Furthermore, 9 (30%) patients experienced a temporary increase of serum creatinine during treatment with CsA, necessitating dose reduction or even stopping of the drug, suggesting that CsA therapy might be difficult to handle in clinical practice.

Mycophenolate mofetil (MMF) was introduced as an effective immunosuppressive agent in transplant patients. The drug caused few side effects. The efficacy has since been demonstrated in patients with SLE, and some studies have suggested equipotency compared with cyclophosphamide. Because cyclophosphamide and azathioprine are considered effective in patients with iMN it seems logical to consider MMF in these patients. Thus far, the experience with MMF in patients with iMN is limited. Briggs *et al.* were the first to report the experience with MMF in patients with iMN.[99] They have treated 3 patients with iMN who had experienced a relapse of proteinuria after withdrawal of other agents. All three patients had normal renal function, and developed a partial remission of proteinuria. After this report the investigators have continued treatment with MMF in patients with iMN. Their experience has been published in detail.[78] The study included 17 patients with iMN and proteinuria. Only 6 patients had evidence of renal insufficiency. Treatment consisted of MMF 0.5 – 1.0 g twice daily for 12 (4 –25) months, combined with steroids in most patients. Overall, proteinuria decreased, and in 2 patients a complete remission (< 0.2 g/day) and in 5 a partial remission (< 2 g/day) was noted. Renal function improved in 3 of 6 patients with renal failure. The heterogeneity of the patients precludes us to draw hard conclusions. In another study Miller *et al.* have treated 16 patients with iMN, the majority with evidence of renal failure, thus high-risk patients.[80] MMF was used in dosages of 500 - 2000 mg/day, and only 5 patients were administered steroids concomitantly. Therapy was continued for only 8 (2-10) months. A partial remission of proteinuria was achieved in 2 patients.

We have also evaluated the effects of MMF in a pilot study in patients with iMN and renal insufficiency.[100] Our treatment regimen consisted of MMF in a dosage of 1000 mg twice daily, combined with steroids as in our cyclophosphamide protocol.

We observed a significant decrease in serum creatinine and proteinuria, in contrast to the findings of Miller *et al.* (Figure 5). The differences in efficacy most likely are explained by the differences in dose and duration of MMF therapy (we have consistently used 2000 mg/day for one year) and the use of steroids (all our patients were administered methylprednisolone pulses and oral prednisone according to our schedule with cyclophosphamide). Although we consider our data as promising, data are too limited to advise the regular use of MMF as standard therapy in patients with iMN.

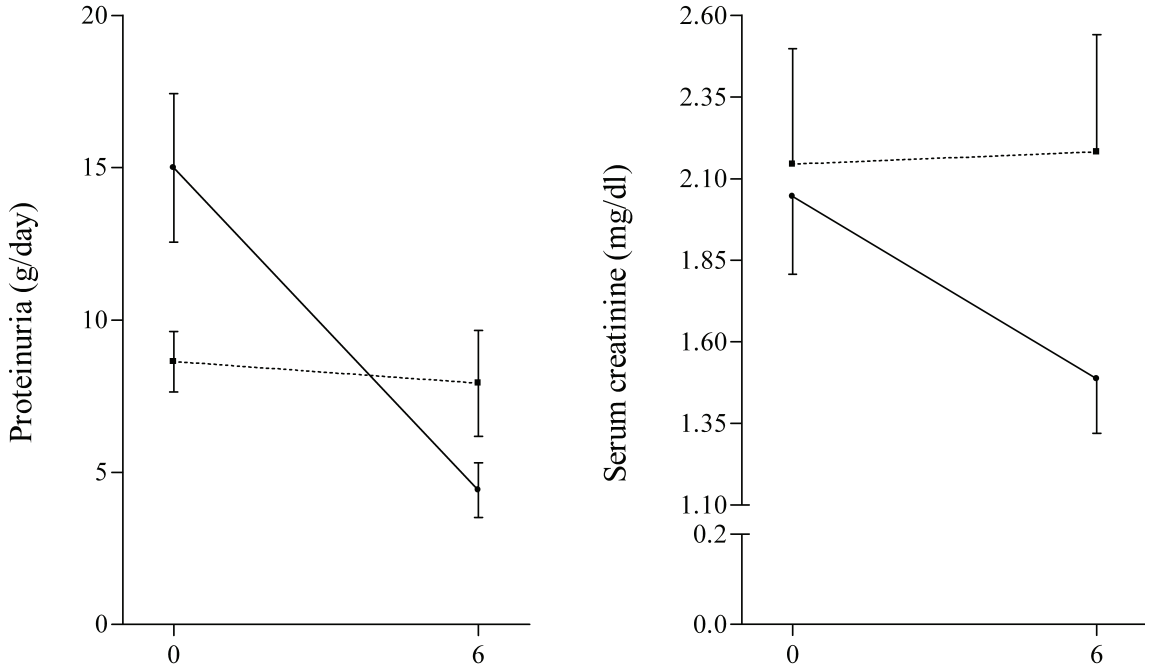


Figure 5. Efficacy of Mycophenolate mofetil in patients with idiopathic membranous nephropathy.

Serum creatinine at baseline and after 6 months are shown as reported by Miller *et al.*[80] (dotted lines) and Du Buf *et al.* [100] (straight lines). The differences are significant, which are likely explained by the higher dose of MMF, the longer duration of therapy and the concomitant use of prednisone in all patients in the latter study.

The anti-CD20 antibody rituximab has proved effective in the treatment of B cell lymphomas. The effectiveness of this agent in decreasing the number of B cells and attenuating antibody production has led to the introduction of this drug in such immune-mediated kidney diseases as iMN. Remuzzi *et al.* have described the results after one year of treatment.[81;101] They have treated 8 patients (3M, 5F) with iMN and a nephrotic syndrome. Renal function was normal in 5 patients. During follow-up, creatinine remained stable and proteinuria decreased to some extent; however only 3 patients developed a partial remission and no patient developed a complete remission. Thus, this study has included low-risk patients (women and patients with

normal renal function) and follow-up has been too short to allow meaningful conclusions on the efficacy of the drug. Subsequent data published in abstract form even suggested that the efficacy may be very limited.[102] In this abstract it was shown that rituximab was ineffective in patients with tubulo-interstitial injury; a decrease in proteinuria was only observed in patients without tubulo-interstitial injury. Because the latter patients usually develop spontaneous remissions these data suggest that rituximab is ineffective in patients at risk for ESRD.

The potential use of ACTH (corticotropin) in patients with iMN has received little attention. Long-acting ACTH administered intramuscularly two to three times weekly decreased serum lipids and proteinuria within 8 weeks.[87] Relapses occurred after ending treatment. However, continued treatment for one year in 5 patients resulted in improvement in renal function and remission of proteinuria. Unfortunately, these data have not been confirmed; effectiveness has only been evaluated in patients with recent-onset iMN, normal renal function and moderate proteinuria.[88]

It generally is accepted that the complement system is involved in iMN. Activation of the complement system with the formation of the C5b-9 membrane attack complex is held responsible for the podocyte injury and proteinuria. The development of a monoclonal antibody directed at C5a held the promise of rational treatment targeting one of the effector molecules. A humanized antibody has allowed studies of patients with iMN. The first study demonstrated no obvious benefits.[86] Admittedly, this may have been caused by the inability of the regimen used to continuously block complement generation. Thus, additional studies are needed to better define the adequate timing of drug administration.

5. Which parameters can be used to identify patients at risk for disease progression?

Ideally, immunosuppressive therapy should be restricted to patients with iMN who will develop ESRD. In such patients, treatment preferably should be started before severe renal insufficiency has developed. Admittedly, there is still no evidence that an early start of treatment will lead to a better preservation of renal function. However, early start of treatment will decrease the time patients spend in a nephrotic phase, with its associated risks for thrombo-embolic complications and premature vascular disease.[29;30;35]

To allow an early start of treatment it is necessary to be able to identify patients at risk for ESRD with high sensitivity and specificity. If a prognostic marker is used to guide treatment, its specificity must be high, preferably > 90%. In such a case, less than 10% of patients will receive treatment unnecessarily. Furthermore, sensitivity also must be high to ensure that patients are indeed detected.

In the literature, several risk markers have been identified that are associated with disease progression. We have reviewed the literature and calculated sensitivities and specificities for the various factors.[49] Examples of risk factors are advanced age, male sex, white race, disturbed renal function at baseline, hypertension, higher glomerular stage and more extensive area of tubulo-interstitial fibrosis. Unfortunately, these parameters lack sufficient sensitivity and specificity and do not allow to guide treatment. In fact, as recent as 1994 Honkanen stated that 'the prediction of renal outcome on clinical basis is hopeless in iMN patients showing a nephrotic syndrome at biopsy'.[37]

Although proteinuria is a well-known predictor of progressive renal injury, the magnitude of proteinuria at baseline is not very discriminative.[73] This is explained readily by the lack of an association between the level of proteinuria and the extent of tubulo-interstitial injury in renal biopsies.[33] By combining the magnitude and the duration of proteinuria, the risk of renal function deterioration can be better estimated. Pei *et al.* observed a 47% risk of progression in patients with a proteinuria of > 4 g for > 18 months, and a 66% risk in patients with > 8 g proteinuria for > 6 months.[103] Sensitivity and specificity improved when using a model that included the level of proteinuria during the 6-month period with greatest proteinuria, as well as serum creatinine at the start of this period and the change in creatinine clearance during this 6-month period (Table 5).[104] Some investigators have advocated measurement of urinary complement products, based on the hypothesis that renal injury in patients with iMN is mediated by complement.[105] Initial studies have suggested a high sensitivity and specificity (Table 5).[106] We and others have studied the predictive value of specific urinary proteins, such as IgG (as marker of glomerular size selectivity) and the low molecular weight proteins β_2-microglobulin (β_2m) or α_1-microglobulin (markers of tubulo-interstitial injury).[11;12;107;108] Both urinary IgG excretion and urinary excretion of low molecular weight proteins proved valuable markers (Table 5).

Table 5. Overview of accuracy of predictors of ESRD in patients with idiopathic membranous nephropathy

Original study *Validation study*	Parameter and threshold	Original study		*Validation study*	
		Sensitivity	Specificity	*Sensitivity*	*Specificity*
Pei[103] *Cattran[104]*	Proteinuria > 8 g > 6 months	66	88	 *58*	 *93*
Cattran[104] *Italy[104]* *Finland[104]*	Model proteinuria and serum creatinine	83	86	 *60* *77*	 *92* *89*
Brenchley[106]	UC3dg > 25 U/mg creat	80	81	 *NA*	 *NA*
Brenchley[106] *Cattran[110]*	UC5b-9 > 7 U/mg creat	60	86	 *Invalid* *See comments in text*	
Reichert[12] *Branten[18]*	UIgG > 250 mg/24 h	89	85	 *88*	 *88*
Bazzi[107]	UIgG > 110 mg/g creat	100	58	 *NA*	 *NA*
Reichert[11] *Branten[18]*	Uβ_2m > 0.5 μg/min	85	82	 *88*	 *91*
Bazzi[107]	Uα_1m > 33 mg/g creat	100	84	 *NA*	 *NA*
Branten[18]	Uα_1m > 40 μg/min	84	94	 *NA*	 *NA*
Reichert[49] *Honkanen[37]*	Serum creatinine > 1.5 mg/dl*	52	90	 *80*	 *92*

* threshold for renal insufficiency has varied from 1.2 to 1.8 mg/dl. NA, not available.

It is important to realize that initial studies often provide a too optimistic view of the value of risk markers. To evaluate these risk markers, their accuracy at the predefined threshold values must be validated in a new patient cohort. To date, the accuracy of urinary complement C3d has not been validated. We have measured urinary C3d in patients with various renal diseases.[109] We observed a good correlation between urinary C3d and the urinary excretion of IgG and β_2m. We calculated that the urinary C3d level was determined by tubular reabsorption processes, as well as glomerular permeability of C3 and local production of C3 and C3d. When corrected for proteinuria there were no differences between patients with iMN and patients with other glomerular diseases. We have not evaluated the prognostic accuracy of C3d, although we expect that urinary C3d will be predictive in view of the good correlation

with IgG and β_2m. However, measurement of urinary C3d is difficult in routine clinical practice, and requires special sampling conditions (in EDTA-containing tubes, placed on ice, centrifuged in the cold and stored at –70 ^{o}C). Furthermore, C3 may interfere in the assay, and the coefficient of variation is 7 – 10%.

The accuracy of the urinary C5b-9 membrane attack complex has not been formally validated. However, Cattran *et al.* measured urine C5b-9 in patients with iMN that had participated in the CsA controlled trial.[110] Notably, urinary C5b-9 was not measurable in the majority of patients (11 out of 16). Furthermore, the absence or presence of the membrane attack complex did not predict outcome or treatment response in their patients.

The change of serum creatinine over a period of 2 years proved a very specific marker (Table 5).[37] Unfortunately, sensitivity is low, and the use of this parameter does not allow the start of treatment before the onset of renal failure.

The model developed by Cattran's group has been validated in a Finnish and Italian population.[104] This validation study proved the high specificity and sensitivity of this model (Table 5). Of note, the validation cohort consisted of treated and untreated patients, and also included patients with non-nephrotic proteinuria (17 – 23%). It is unclear whether results would have been similar if the validation cohorts had only included untreated patients with a nephrotic syndrome. Furthermore, application of the model requires a period of follow-up to identify the 6-month period with the highest level of persistent proteinuria. In approximately one quarter of patients in the validation study, the period of maximal persistent proteinuria started $>$ 12 months after renal biopsy.

We recently have validated the use of urinary IgG and β_2m in a new cohort of patients with iMN.[18] The data unequivocally proved that these markers predict prognosis, with sensitivities and specificities approximating 90%. Specificity approached 100% when combining urinary β_2m and serum albumin. In Figure 6, renal survival is depicted for patients with urinary β_2m and serum albumin levels less than or greater than the threshold.

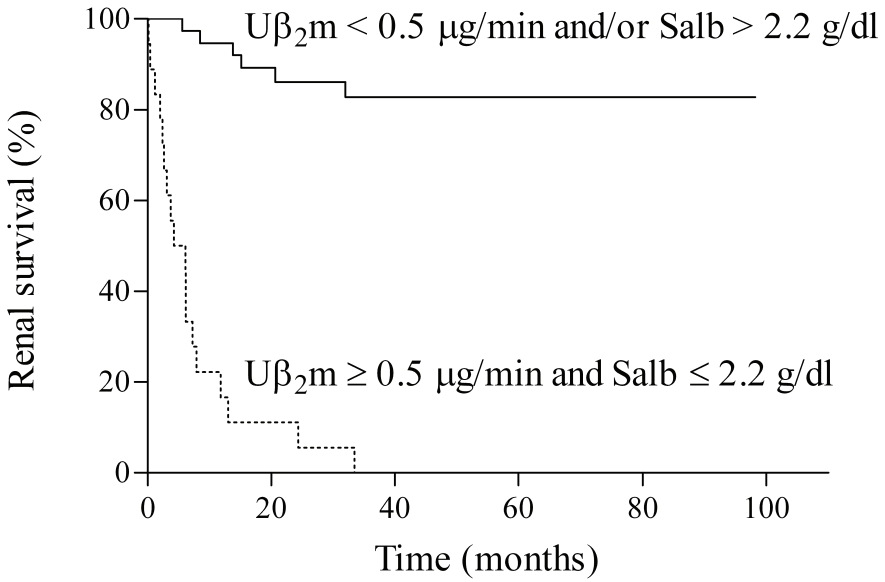


Figure 6. Urinary β_2-microglobulin excretion and serum albumin predict renal survival in patients with idiopathic membranous nephropathy, a nephrotic syndrome and normal renal function at biopsy.

Data adapted from Branten *et al*.[18]
Threshold values were 0.5 μg/min for urinary β_2m and 2.2 g/dl for serum albumin.

The use of the Toronto model or specific urinary protein analysis should allow us to restrict therapy to patients at greatest risk for disease progression. We prefer measurements of low molecular weight proteins rather than the duration and magnitude of proteinuria, because of the greater accuracy, its easy applicability (no need for 24-h urine collections) and its direct use (no need for a waiting period).

In conclusion, our treatment strategy is intended to allow individualized treatment for patients with iMN. High-risk patients can be identified readily. Patients at risk for developing ESRD should receive immunosuppressive therapy. Currently, we prefer a combination of cyclophosphamide and steroids. Alternative agents include CsA and MMF, however their efficacy on long term remains to be proved.

References

1. Haas M, Meehan SM, Karrison TG, Spargo BH. Changing etiologies of unexplained adult nephrotic syndrome: A comparison of renal biopsy findings from 1976-1979 and 1995-1997. *Am J Kidney Dis* 1997; 30: 621-631
2. Schieppati A, Mosconi L, Perna A, *et al*. Prognosis of untreated patients with idiopathic membranous nephropathy. *New Engl J Med* 1993; 329: 85-89
3. Ponticelli C, Zuchelli P, Passerini P, *et al*. A 10-year follow-up of a randomized study with methylprednisolone and chlorambucil in membranous nephropathy. *Kidney Int* 1995; 48: 1600-1604
4. Cameron JS. Membranous nephropathy: the treatment dilemma. *Am J Kidney Dis* 1982; 1: 371-375
5. Cameron JS. Membranous nephropathy - still a treatment dilemma. *N Engl J Med* 1992; 327: 638-639
6. Glassock RJ. The treatment of idiopathic membranous nephropathy: a dilemma or a conundrum? *Am J Kidney Dis* 2004; 44: 562-566
7. Perna A, Schieppati A, Zamora J, *et al*. Immunosuppressive treatment for idiopathic membranous nephropathy: a systematic review. *Am J Kidney Dis* 2004; 44: 385-401
8. du Buf-Vereijken PWG, Branten AJW, Wetzels JFM, for the Membranous Nephropathy Study Group. Cytotoxic therapy for membranous nephropathy and renal insufficiency; improved renal survival but high relapse rate. *Nephrol Dialysis Transplant* 2004; 19: 1142-1148
9. Torres A, Dominguez-Gil B, Carreno A, *et al*. Conservative versus immunosuppressive treatment of patients with idiopathic membranous nephropathy. *Kidney Int* 2002; 61: 219-227
10. Reichert LJM, Huysmans FThM, Assmann K, Koene RAP, Wetzels JFM. Preserving renal function in patients with membranous nephropathy: daily oral chlorambucil compared with intermittent monthly pulses of cyclophosphamide. *Ann Intern Med* 1994; 121: 328-333
11. Reichert LJM, Koene RAP, Wetzels JFM. Urinary excretion of β_2-microglobulin predicts renal outcome in patients with idiopathic membranous nephropathy. *J Am Soc Nephrol* 1995; 6: 1666-1669
12. Reichert LJM, Koene RAP, Wetzels JFM. Urinary IgG excretion as a prognostic factor in idiopathic membranous nephropathy. *Clin Nephrol* 1997; 48: 79-84
13. Wetzels JFM, Reichert LJM. Efficacy of immunosuppressive treatment in patients with membranous nephropathy and renal insufficiency. *Kidney Int* 1997; 52 Suppl 61: S63-S66
14. Branten AJW, Reichert LJM, Koene RAP, Wetzels JFM. Oral cyclophosphamide versus chlorambucil in the treatment of patients with idiopathic membranous nephropathy and renal insufficiency. *Q J Med* 1998; 91: 359-366
15. Branten AJW, Wetzels JFM. Short- and long-term efficacy of oral cyclophosphamide and steroids in patients with membranous nephropathy and renal insufficiency. *Clin Nephrol* 2001; 56: 1-8
16. du Buf-Vereijken PWG, Feith GW, Hollander D, *et al*. Restrictive use of immunosuppressive treatment in patients with idiopathic membranous nephropathy: high renal survival in a large patient cohort. *Q J Med* 2004; 97: 353-360

17. du Buf-Vereijken PWG, Wetzels JFM. Efficacy of a second course of immunosuppressive therapy in patients with membranous nefropathy and persistent or relapsing disease activity. *Nephrol Dialysis Transplant* 2004; 19: 2036-2043

18. Branten AJW, du Buf-Vereijken PWG, Klasen IS, *et al*. Urinary excretion of β_2-microglobulin and IgG predict prognosis in idiopathic membranous nephropathy: a validation study. *J Am Soc Nephrol* 2005; 16: 169-174

19. Erwin DT, Donadio JV, Holley KE. The clinical course of idiopathic membranous nephropathy. *Mayo Clin Proc* 1973; 48: 697-712

20. Ehrenreich T, Porush JG, Churg J, *et al*. Treatment of idiopathic membranous nephropathy. *N Engl J Med* 1976; 295: 741-746

21. Bolton WK, Atuk N, Sturgill BC, Westervelt FB. Therapy of the idiopathic nephrotic syndrome with alternate day steroids. *Am J Med* 1977; 62: 60-70

22. Gluck MC, Gallo G, Lowenstein J, Baldwin DS. Membranous glomerulonephritis: evolution of clinical and pathological features. *Ann Intern Med* 1973; 16: 13-19

23. Pierides AM, Malasit P, Morley AR, *et al*. Idiopathic membranous nephropathy. *Q J Med* 1977; 182: 163-177

24. Franklin WA, Jennings RB, Earle DP. Membranous glomerulonephritis: long-term serial observations on clinical course and morphology. *Kidney Int* 1973; 4: 36-56

25. Row PG, Cameron JS, Turner DR, *et al*. Membranous nephropathy; long-term follow-up and association with neoplasia. *Q J Med* 1975; 174: 207-239

26. Forland M, Spargo BH. Clinicopathological correlation in idiopathic nephrotic syndrome with membranous nephropathy. *Nephron* 1969; 6: 498-525

27. Collaborative study of the adult idiopathic nephrotic syndrome. A controlled study of short-term prednisone treatment in adults with membranous nephropathy. *New Engl J Med* 1979; 301: 1301-1306

28. Davison AM, Cameron JS, Kerr DN, Ogg CS, Wilkinson RW. The natural history of renal function in untreated idiopathic membranous glomerulonephritis in adults. *Clin Nephrol* 1984; 22: 61-67

29. MacTier R, Boulton Jones JM, Payton CD, McLay A. The natural history of membranous nephropathy in the West of Scotland. *Q J Med* 1986; 60: 793-802

30. Zuchelli P, Ponticelli C, Gagnoli P, Passerini P. Long-term outcome of idiopathic membranous nephropathy with nephrotic syndrome. *Nephrol Dialysis Transplant* 1987; 2: 73-78

31. Donadio JJV, Torres VE, Velosa JA, *et al*. Idiopathic membranous nephropathy: The natural history of untreated patients. *Kidney Int* 1988; 33: 708-715

32. Cattran DC, Delmore T, Roscoe J, *et al*. A randomized controlled trial of prednisone in patients with idiopathic membranous nephropathy. *New Engl J Med* 1989; 320: 210-215

33. Wehrmann M, Bohle A, Bogenschutz O, *et al*. Long-term prognosis of chronic idiopathic membranous glomerulonephritis. An analysis of 334 cases with particular regard to tubulo-interstitial changes. *Clin Nephrol* 1989; 31: 67-76

34. Cameron JS, Healy MJR, Adu D. The Medical Research Council trial of short-term high-dose alternate day prednisolone in idiopathic membranous nephropathy with nephrotic syndrome in adults. *Q J Med* 1990; 74: 133-156

35. Durin S, Barbanel C, Landais P, Noel LH, Grunfeld JP. Long term course of idiopathic extramembranous glomerulonephritis. Study of predictive factors of terminal renal insufficiency in 82 untreated patients. *Nephrologie* 1990; 11: 67-71

36. Noel LH, Zanetti M, Droz D. Long-term prognosis of idiopathic membranous glomerulonephritis. *Am J Med* 1979; 66: 82-90

37. Honkanen E, Tornroth T, Gronhagen-Riska C, Sankila R. Long-term survival in idiopathic membranous glomerulonephritis: can the course be clinically predicted? *Clin Nephrol* 1994; 41: 127-134

38. Laluck BJ, Cattran DC. Prognosis after a complete remission in adult patients with idiopathic membranous nephropathy. *Am J Kidney Dis* 1999; 33: 1026-1032

39. Yoshimoto K, Yokoyama H, Wada T, *et al*. Pathologic findings of initial biopsies reflect the outcomes of membranous nephropathy. *Kidney Int* 2004; 65: 148-153

40. Lewis EJ, Hunsicker LG, Bain RP, Rohde RD. The effect of angiotensin-converting-enzyme inhibition on diabetic nephropathy. The Collaborative Study Group. *N Engl J Med* 1993; 329: 1456-1462

41. Brenner BM, Cooper ME, de Zeeuw D, *et al*. Effects of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy. *N Engl J Med* 2001; 345: 861-869

42. Jafar TH, Schmid CH, Landa M, *et al*. Angiotension-converting enzyme inhibitors and progression of nondiabetic renal disease. A meta-analysis of patient-level data. *Ann Intern Med* 2001; 135: 73-87

43. The Gisen Group. Randomised placebo-controlled trial of effect of ramipril on decline in glomerular filtration rate and risk of terminal renal failure in proteinuric, non-diabetic nephropathy. *Lancet* 1997; 349: 1857-1863

44. Stegeman CA, de Zeeuw D, de Jong PE. Treatment of idiopathic membranous nephropathy: the dilemma of who, when, and how. *Nephrol Dialysis Transplant* 1995; 10: 1982-1984

45. Troyanov S, Wall CA, Miller JA, *et al*. Idiopathic membranous nephropathy: definition and relevance of a partial remission. *Kidney Int* 2004; 66: 1199-1205

46. Praga M, Borstein B, Andres A, *et al*. Nephrotic proteinuria without hypoalbuminemia: clinical characteristics and response to angiotensin-converting enzyme inhibition. *Am J Kidney Dis* 1991; 17: 330-338

47. Praga M, Hernandez E, Montoyo C, *et al*. Long-term beneficial effects of angiotensin-converting enzyme inhibition in patients with nephrotic proteinuria. *Am J Kidney Dis* 1992; 20: 240-248

48. Ponticelli C, Passerini P, Altieri P, Locatelli F, Pappalettera M. Remissions and relapses in idiopathic membranous nephropathy. *Nephrol Dialysis Transplant* 1992; 7 Suppl 1: 85-90

49. Reichert LJM, Koene RAP, Wetzels JFM. Prognostic factors in idiopathic membranous nephropathy. *Am J Kidney Dis* 1998; 31: 1-11

50. Passerini P, Pasquali P, Cesana B, Zucchelli P, Ponticelli C. Long-term outcome of patients with membranous nephropathy after complete remission of proteinuria. *Nephrol Dialysis Transplant* 1989; 4: 525-529

51. Rastogi SP, Hart-Mercer J, Kerr DN. Idiopathic membranous glomerulonephritis in adults: remission following steroid therapy. *Q J Med* 1969; 38: 335-350

52. Hopper Jr. J, Biava CG, Tu W-H. Membranous nephropathy: high dose alternate-day therapy with prednisone. *West J Med* 1981; 135: 1-8

53. Short CD, Solomon LR, Gokal R, Mallick NP. Methylprednisolone in patients with membranous nephropathy and declining renal function. *Q J Med* 1987; 247: 929-940

54. Fuiano G, Stanziale P, Balletta M, *et al*. Effectiveness of steroid therapy in different stages of membranous nephropathy. *Nephrol Dialysis Transplant* 1989; 4: 1022-1029

55. Brunkhorst R, Wrenger E, Koch KM. Low-dose prednisolone/chlorambucil therapy in patients with severe membranous glomerulonephritis. *Clin Invest* 1994; 72: 277-282

56. Bruns FJ, Adler S, Fraley DS, Segel DP. Sustained remission of membranous glomerulonephritis after cyclophosphamide and prednisone. *Ann Intern Med* 1991; 114: 725-730

57. Faedda R, Satta A, Bosincu L, Pirisi M, Bartoli E. Immune suppressive treatment of membranous glomerulonephritis. *J Nephrol* 1995; 8: 107-112

58. Falk RJ, Hogan SL, Muller KE, Jennette JC, Glomerular disease collaborative network. Treatment of progressive membranous glomerulopathy. *Ann Intern Med* 1992; 116: 438-445

59. Jindal K, West M, Bear R, Goldstein M. Longterm benefits of therapy with cyclophosphamide and prednisone in patients with membranous glomerulonephritis and impaired renal function. *Am J Kidney Dis* 1992; 19: 61-67

60. Mathieson PW, Turner AN, Maidment CG, Evans DJ, Rees AJ. Prednisolone and chlorambucil treatment in idiopathic membranous nephropathy with deteriorating renal function. *Lancet* 1988; 2: 869-872

61. Ponticelli C, Zuchelli P, Passerini P, *et al*. A randomized trial of methylprednisolone and chlorambucil in idiopathic membranous nephropathy. *New Engl J Med* 1989; 320: 8-13

62. Ponticelli C, Zuchelli P, Imbasciati E, *et al*. Controlled trial of methylprednisolone and chlorambucil in idiopathic membranous nephropathy. *New Engl J Med* 1984; 310: 946-950

63. Warwick GL, Geddes CG, Boulton-Jones JM. Prednisolone and chlorambucil therapy for idiopathic membranous nephropathy with progressive renal failure. *Q J Med* 1994; 87: 223-229

64. Warwick GL, Boulton-Jones JM. Immunosuppression for membranous nephropathy. *Lancet* 1988; I: 1361 (Letter)

65. Ford AR. Improvement of renal failure due to membranous glomerulonephritis after unsuccessful renal transplantation. *Nephron* 1978; 20: 304-306

66. Murphy BF, McDonald I, Fairley KF, Kincaid-Smith PS. Randomized controlled trial of cyclophosphamide, warfarin and dipyridamole in idiopathic membranous glomerulonephritis. *Clin Nephrol* 1992; 37: 229-234

67. Donadio JJV, Holley KE, Anderson CF, Taylor WF. Controlled trial of cyclophosphamide in idiopathic membranous nephropathy. *Kidney Int* 1974; 6: 431-439

68. Braun N, Erley CM, Benda N, *et al*. Therapy of membranous glomerulonephritis with nephrotic syndrome: 5 years follow-up of a prospective, randomized multi-center study. *J Am Soc Nephrol* 1995; 6: 413 (Abstract)

69. Risler T, Braun N, Bach D, *et al*. The German glomerulonephritis therapy study: 10 years of controlled randomized trials for the treatment of idiopathic glomerulonephritis. *Kidney Blood Press Res* 1996; 19: 196-200

70. Wetzels JFM, Hoitsma AJ, Koene RAP. Immunosuppression for membranous nephropathy. *Lancet* 1989; I: 211-211 (Letter)

71. Ahuja M, Goumenos D, Shortland JR, Gerakis A, Brown CB. Does immunosuppression with prednisolone and azathioprine alter the progression of idiopathic membranous nephropathy? *Am J Kidney Dis* 1999; 34: 521-529

72. Alexopoulos E, Sakellariou G, Memmos D, *et al*. Cyclophosphamide provides no additional benefit to steroid therapy in the treatment of idiopathic membranous nephropathy. *Am J Kidney Dis* 1993; 21: 497-503

73. Bone JM, Rustom R, Williams PS. "Progressive" versus "indolent" idiopathic membranous glomerulonephritis. *Q J Med* 1997; 90: 699-706

74. Baker LRI, Tucker B, Macdougall IC. Treatment of idiopathic membranous nephropathy. *Lancet* 1994; 343: 290-291 (Letter)

75. Brown JH, Alistair FD, Murphy BG, *et al*. Treatment of renal failure in idiopathic membranous nephropathy with azathioprine and prednisolone. *Nephrol Dialysis Transplant* 1998; 13: 443-448

76. Cattran DC, Greenwood C, Ritchie S, *et al*. A controlled trial of cyclosporine in patients with progressive membranous nephropathy. *Kidney Int* 1995; 47: 1130-1135

77. Cattran DC, Appel GB, Hebert LA, *et al*. Cyclosporine in patients with steroid-resistant membranous nephropathy: a randomized trial. *Kidney Int* 2001; 59: 1484-1490

78. Choi MJ, Eustace JA, Gimenez LF, *et al*. Mycophenolate mofetil treatment for primary glomerular diseases. *Kidney Int* 2002; 61: 1098-1114

79. Guasch A, Suranyi M, Newton L, Hall BM, Myers BD. Short-term responsiveness of membranous glomerulopathy to cyclosporine. *Am J Kidney Dis* 1992; 20: 472-481

80. Miller G, Zimmerman III R, Radhakrishnan J, Appel GB. Use of mycophenolate mofetil in resistant membranous nephropathy. *Am J Kidney Dis* 2000; 36: 250-256

81. Remuzzi G, Chiurchiu C, Abbate M, *et al*. Rituximab for idiopathic membranous nephropathy. *Lancet* 2002; 360: 923-924

82. Rostoker G, Belghiti D, Maadi A-B, *et al*. Long-term cyclosporin A therapy for severe idiopathic membranous nephropathy. *Nephron* 1993; 63: 335-341

83. Stirling CM, Simpson K, Boulton-Jones JM. Immunosuppression and outcome in idiopathic membranous nephropathy. *Q J Med* 1998; 9: 159-164

84. Western Canadian Glomerulonephritis Study Group. Controlled trial of azathioprine in the nephrotic syndrome secondary to idiopathic membranous glomerulonephritis. *C M J* 1976; 115: 1209-1210

85. Williams PS, Bone JM. Immunosuppression can arrest progressive renal failure due to idiopathic membranous glomerulonephritis. *Nephrol Dialysis Transplant* 1989; 4: 181-186

86. Appel G, Nachman P, Hogan SL, *et al*. Eculizumab (C5 complement inhibitor) in the treatment of idiopathic membranous nephropathy: preliminary baseline and pharmacokinetic/pharmacodynamic data. *J Am Soc Nephrol* 2002; 13: 668A (Abstract)

87. Berg AL, Nilsson-Ehle P, Arnadottir M. Beneficial effects of ACTH on the serum lipoprotein profile and glomerular function in patients with membranous nephropathy. *Kidney Int* 1999; 56: 1534-1543

88. Picardi L, Villa G, Galli F, *et al*. ACTH therapy in nephrotic syndrome induced by idiopathic membranous nephropathy. *Clin Nephrol* 2004; 62: 403-404

89. Ponticelli C, Altieri P, Scolari F, *et al*. A randomized study comparing methylprednisolone plus chlorambucil versus methylprednisolone plus cyclophophamide in idiopathic membranous nephropathy. *J Am Soc Nephrol* 1998; 9: 444-450

90. Jayne D, Rasmussen N, Andrassy K, *et al*. A randomized trial of maintenance therapy for vasculitis associated with antineutrophil cytoplasmic antibodies. *N Engl J Med* 2003; 349: 36-44

91. Wetzels JFM. Cyclophosphamide-induced gonadal toxicity: a treatment dilemma in patients with lupus nephritis? *Neth J Med* 2004; 62: 347-352

92. Zietse R, Wenting GJ, Kramer P, *et al*. Contrasting response to cyclosporin in refractory nephrotic syndrome. *Clin Nephrol* 1989; 31: 22-25

93. Schrijver G, Wetzels JFM, Robben JCM, *et al*. Antiproteinuric effect of Cyclosporin A in passive antiglomerular basement membrane nephritis in the mouse. *Transplant Proc* 1988; 20(suppl 4): 304-308

94. Schrijver G, Assmann KJM, Wetzels JFM, Berden JHM. Cyclosporin A reduces albuminuria in experimental anti-GBM glomerulonephritis independently from changes in GFR. *Nephrol Dialysis Transplant* 1995; 10: 1149-1154

95. Zietse R, Wenting GJ, Kramer P, Schalekamp MA, Weimar W. Effects of cyclosporine on glomerular barrier function in nephrotic syndrome. *Clin Sci* 1992; 82: 641-650

96. Ambalavanan S, Fauvel JP, Sibley RK, Myers BD. Mechanisms of the antiproteinuric effect of cyclosporine in membranous nephropathy. *J Am Soc Nephrol* 1996; 7: 290-298

97. Pisoni R, Grinyo JM, Salvadori M, *et al*. Cyclosporine versus conservative therapy in patients with idiopathic membranous nephropathy and deteriorating renal function: results of the CYCLOMEN trial. *J Am Soc Nephrol* 2000; 11: A0514 (Abstract)

98. Ponticelli C, Villa M. Does cyclosporin have a role in the treatment of membranous nephropathy? *Nephrol Dialysis Transplant* 1999; 14: 23-25

99. Briggs WA, Choi MJ, Scheel PJ, Jr. Successful mycophenolate mofetil treatment of glomerular disease. *Am J Kidney Dis* 1998; 31: 213-217

100. du Buf-Vereijken PW, Wetzels JFM. Mycophenolate mofetil versus cyclophosphamide in patients with idiopathic membranous nephropathy and renal insufficiency. *J Am Soc Nephrol* 2004; 15: 341A (Abstract)

101. Ruggenenti P, Chiurchiu C, Brusegan V, *et al*. Rituximab in idiopathic membranous nephropathy: a one-year prospective study. *J Am Soc Nephrol* 2003; 14: 1851-1857

102. Ruggenenti P, Chiurchiu C, Brusegan V, *et al*. Rituximab for idiopathic membranous nephropathy: renal biopsy findings predict response to treatment. *J Am Soc Nephrol* 2003; 14: 528A (Abstract)

103. Pei Y, Cattran DC, Greenwood C. Predicting chronic renal insufficiency in idiopathic membranous glomerulonephritis. *Kidney Int* 1992; 42: 960-966

104. Cattran DC, Pei Y, Greenwood CMT, *et al*. Validation of a predictive model of idiopathic membranous nephropathy: its clinical and research implications. *Kidney Int* 1997; 51: 901-907

105. Kon SP, Coupes B, Short CD, *et al*. Urinary C5b-9 excretion and clinical course in idiopathic membranous nephropathy. *Kidney Int* 1995; 48: 1953-1958

106. Brenchley PE, Coupes B, Short CD, *et al*. Urinary C3dg and C5b-9 indicate active immune disease in human membranous nephropathy. *Kidney Int* 1992; 41: 933-937

107. Bazzi C, Petrini C, Rizza V, *et al*. Urinary excretion of IgG and alpha$_1$-microglobulin predicts clinical course better than extent of proteinuria in membranous nephropathy. *Am J Kidney Dis* 2001; 38: 240-248

108. Bazzi C, D'Amico G. The urinary excretion of IgG and alpha$_1$-microglobulin predicts renal outcome and identifies patients deserving treatment in membranous nephropathy. *Kidney Int* 2002; 61: 2276

109. Branten AJ, Kock-Jansen M, Klasen IS, Wetzels JF. Urinary excretion of complement C3d in patients with renal diseases. *Eur J Clin Invest* 2003; 33: 449-456

110. Cattran DC, Wald R, Brenchley PE, Coupes B. Clinical correlates of serial urinary membrane attack complex estimates in patients with idiopathic membranous nephropathy. *Clin Nephrol* 2003; 60: 7-12

Chapter 10

Summary

Summary

The use of immunosuppressive therapy in patients with idiopathic membranous nephropathy is heavily debated. We prefer treating only patients at highest risk for disease progression. From 1988 onward, we have applied a restrictive treatment strategy, limiting the use of immunosuppressive drugs to patients with evidence of progressive renal failure. In addition, we have continued the search for risk markers of disease progression and for better immunosuppressive agents. In Figure 1, we provide an overview with reference to the relevant chapters.

We first evaluated the efficacy of immunosuppressive therapy in patients at highest risk for end-stage renal disease: patients with evidence of renal function deterioration (serum creatinine > 135 μmol/l and/or a rise in serum creatinine of > 50%). In ***Chapter 2*** we report the prospective study of 65 patients with idiopathic membranous nephropathy and renal insufficiency, treated with cyclophosphamide and steroids. The treatment schedule consisted of oral cyclophosphamide, 1.5-2.0 mg/kg/day for 12 months and steroids, methylprednisolone pulses 3 x 1 g, i.v. at months 0, 2 and 4 and oral prednisone 0.5 mg/kg/48 h for 6 months. Median follow-up was 51 months. Renal function temporarily improved or stabilized in all patients. A partial remission (PR, proteinuria 0.21-2.0 g/10 mmol creatinine) occurred in 56 patients; 17 patients improved into a complete remission (CR, proteinuria < 0.2 g/10 mmol creatinine). During follow-up, 11 patients relapsed (28% relapse rate after 5 years) to a nephrotic syndrome. Nine of them were re-treated because of renal function deterioration. At the end of follow-up, 16 patients were in CR, 31 in PR, eight had a persistent nephrotic syndrome, one had mild proteinuria, four had progressed to ESRD and five had died. Overall renal survival was 86% after 5 years and 74% after 7 years. To demonstrate the efficacy of immunosuppressive therapy in patients with renal function deterioration, we have compared renal survival in our treated patients with outcome in a group of historical control patients (n=24), who were either not treated or treated with ineffective immunosuppression. Renal survival in this historical control group was only 32% after 5 and 7 years. Unfortunately, immunosuppressive treatment was accompanied by complications in two-thirds of patients. Side effects consisted mainly of bone marrow depression and infections. Furthermore, one patient has developed bladder cancer, a feared complication of cyclophosphamide therapy.

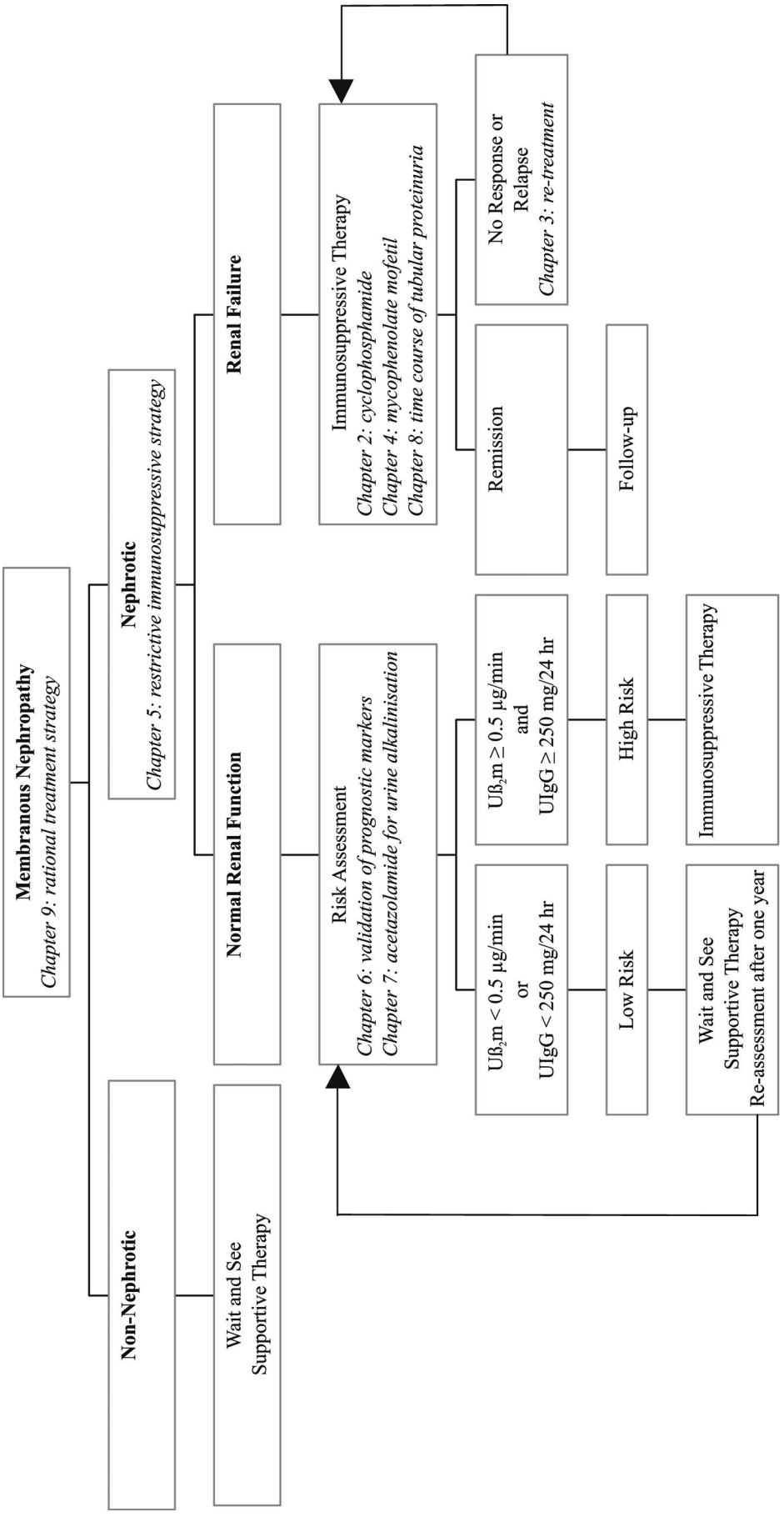


Figure 1. Rational treatment strategy for idiopathic membranous nephropathy.

From this study we conclude that renal survival can be significantly improved by treating patients with idiopathic membranous nephropathy and renal insufficiency with cyclophosphamide and steroids. However, side effects occur frequently and the relapse rate is high during longer follow-up.

In ***Chapter 3*** we have prospectively studied the clinical course in 15 patients, who either did not respond to the first course of immunosuppression or had experienced a relapse of the nephrotic syndrome, accompanied with renal function deterioration. These patients were treated with a consecutive course of immunosuppression. Initial immunosuppression consisted of either chlorambucil or cyclophosphamide and was started 8 months (range: 0-143 months) after renal biopsy. The second course of immunosuppression consisted of cyclophosphamide and steroids in all patients. The interval between the first and second course was 40 months (range: 7-112 months). Total follow-up was 110 months (range: 46-289 months). Renal function and proteinuria improved at least temporarily in all patients after the second course. Four patients have needed an additional course of therapy. At the end of follow-up two patients were in complete remission, eight in partial remission, three patients experienced persistent proteinuria, one patient had progressed to end-stage renal disease and one patient had died. Renal survival was 86% at 5 and 10 years of follow-up. The repeated courses of immunosuppression have resulted in a gain of dialysis-free survival time of more than 93 months (range: 43-192 months). In conclusion, we think that a second course of immunosuppression should be advised to patients with persistent or relapsing nephrotic syndrome and renal insufficiency.

Despite the efficacy of cyclophosphamide, the high rate of immunosuppressive therapy-related side effects prompted us to search for newer treatment strategies, aiming at high efficacy and fewer complications. In ***Chapter 4*** we report the interim results of an ongoing pilot study, which started in May 2002. In this study we have treated patients with renal insufficiency with mycophenolate mofetil (MMF), 1000 mg twice daily for 12 months in combination with steroids in the same schedule as mentioned previously. Thirteen patients have completed the treatment year and these results were compared with 13 matched historical controls treated with cyclophosphamide. MMF and cyclophosphamide were equally effective in improving renal function and reducing proteinuria. In the treatment year, eight (mycophenolate mofetil) and seven (cyclophosphamide) patients developed a partial

remission of proteinuria. Approximately 70% of patients experienced side effects with both treatment schedules. Patients on cyclophosphamide mainly experienced leucocytopenia, whereas patients on mycophenolate mofetil suffered from anemia. Both patient groups experienced infections. Although not less frequent, side effects on mycophenolate mofetil were less severe. Unfortunately, two patients treated with mycophenolate mofetil had a treatment failure. Therefore, the results of treatment of a larger group of patients with longer follow-up have to be awaited before firm conclusions on the efficacy of mycophenolate mofetil for this high-risk patient group can be drawn.

In ***Chapter 5*** we demonstrate the benefits of a restrictive treatment policy. We have prospectively studied a cohort of 69 patients with normal renal function and a nephrotic syndrome at the time of renal biopsy. Immunosuppressive treatment, mainly consisting of cyclophosphamide and steroids, was advised only in patients with renal insufficiency or severe intolerable nephrotic syndrome. At the time of biopsy average serum creatinine was 90 μmol/l and proteinuria 6.7 g/day. After a follow-up of more than 5 years, 33 (48%) patients have received immunosuppressive therapy, mainly because of renal insufficiency (n=24). At the end of follow-up 22 patients were in complete remission (32%), 24 patients in partial remission (35%), 15 patients still had a nephrotic syndrome (22%), one patient experienced mild proteinuria (1.4%), six patients had developed end-stage renal disease (8.7%) and one patient had died. Renal survival was 94% at 5 years and 88% at 7 years. For comparison, Ponticelli *et al.* who treated 100% of patients reported a 10-year survival rate of 92%. We conclude that a restrictive treatment policy assures a favourable prognosis, while preventing exposure to immunosuppressive therapy in > 50% of the patients.

Nevertheless, we cannot exclude that prognosis can be improved by treating patients at high risk for renal function deterioration in an earlier phase, i.e. before serum creatinine rises significantly. To do so, we must be able to identify high-risk patients early in the course of the disease. In ***Chapter 6*** we have validated the formerly suggested value of urinary β_2-microglobulin and IgG in predicting prognosis. In a prospective study, started in 1995, in 58 patients with membranous nephropathy, normal renal function and a nephrotic syndrome, a standardized measurement was carried out to determine renal function and the excretion of low and high molecular weight proteins. The endpoint renal death was sharply defined as a serum creatinine exceeding 135 μmol/l (1.5 mg/dl), a rise of serum creatinine of > 50%, or

need of immunosuppressive treatment. After a follow-up time of more than 4 years, 25 (43%) of the patients had reached the endpoint. Multivariate analysis by Cox proportional hazard confirmed urinary β_2-microglobulin excretion, using the threshold value of 0.5 μg/min, as the strongest independent predictor for the development of renal insufficiency, with a sensitivity of 83% and a specificity of 88%. Sensitivity and specificity were 84% and 85% respectively for the urinary excretion of IgG, applying the threshold value of 250 mg/24 hours. When the excretions of both proteins were combined specificity improved to 94%. Therefore, urinary β_2-microglobulin and IgG are useful markers to guide decisions on the start of immunosuppressive treatment.

Unfortunately, β_2-microglobulin in urine can only be accurately measured in alkaline urine. We therefore use sodium bicarbonate to alkalinize urine to levels of pH above 6.0. However, in 7% of patients, this value cannot be reached. We investigated if administration of acetazolamide, a carbonic anhydrase inhibitor, would sufficiently alkalinize urine, without influencing urinary protein excretion rates. In ***Chapter 7*** we show, that the administration of acetazolamide adequately increased urinary pH, thus allowing measurement of urinary β_2-microglobulin, without influencing the urinary excretion of β_2-microglobulin. However, side effects occurred frequently, mainly consisting of paresthesias (58%), changes in taste (32%) and dizziness (29%). Although these side effects were not severe, they prevent the routine use of acetazolamide.

Given the value of the urinary β_2-microglobulin and IgG excretion in predicting the occurrence of renal function deterioration in patients with normal renal function, we wondered what happened with the urinary excretion of the high and low molecular weight proteins during and after immunosuppressive therapy. In ***Chapter 8*** we investigated the time-course of the urinary excretion of these proteins after start of therapy and evaluated their value in predicting long-term outcome. Eleven patients underwent repeated standardized measurements of urinary IgG, β_2-microglobulin and α_1-microglobulin during the treatment year. We observed a rapid improvement in glomerular perm selectivity and tubular protein reabsorption within 2 months after start of therapy. Despite a partial remission of proteinuria within 12 months in most patients, evidence of tubulo-interstitial injury remained apparent. In order to investigate the predictive value of the urinary excretions of the high and low molecular weight proteins at the end of the treatment year, 25 patients had measurements at

12 months. However, the urinary levels of IgG, β_2-microglobulin and α_1-microglobulin neither at baseline nor at 12 months clearly predicted the occurrence of a remission or a relapse to nephrotic range proteinuria. Seventeen patients had measurements 2-5 years after the start of immunosuppressive treatment. In case of a persistent stable remission we observed a gradual decrease in urinary β_2-microglobulin towards normal values, whereas in case of a relapse to nephrotic range proteinuria urinary β_2-microglobulin excretion increased. The data of this study allowed us to investigate the relationship between the tubular reabsorption of β_2-microglobulin and the urinary excretion of IgG. We could conclude that the urinary excretion of β_2-microglobulin is not the result of inhibition of its reabsorption by IgG, thus confirming the value of β_2-microglobulin as a marker of tubulo-interstitial injury.

In ***Chapter 9***, we propose a rational treatment strategy for patients with idiopathic membranous nephropathy based on a review of the literature and our own experience. The proposed strategy is depicted in Figure 1.

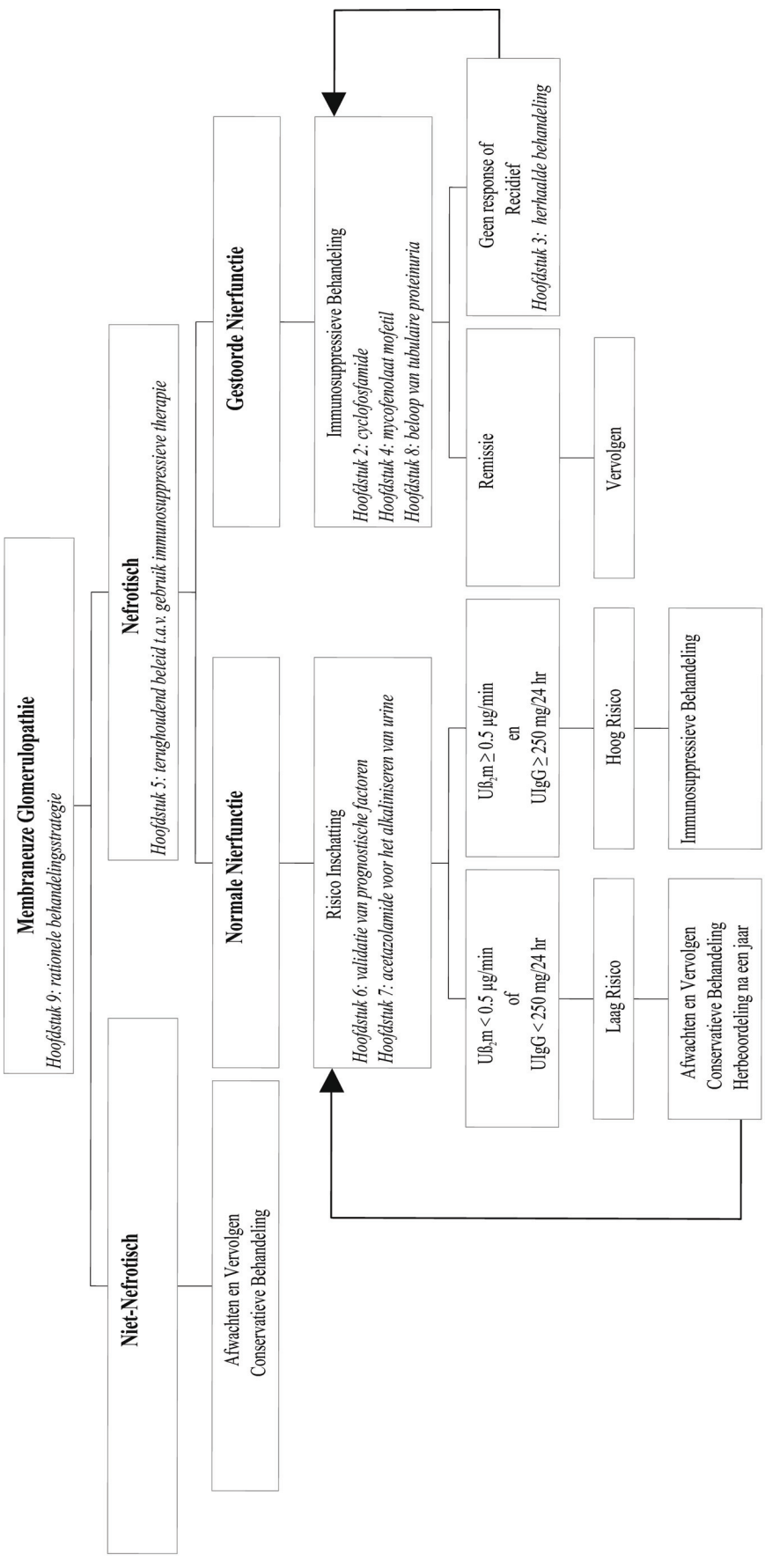
Chapter 11

Samenvatting

Samenvatting

Er is geen eenduidig beleid t.a.v. de immunosuppressieve behandeling van patiënten met een idiopathische membraneuze glomerulopathie. Wij geven er de voorkeur aan om alleen patiënten met het hoogste risico op progressie (achteruitgang) van de ziekte te behandelen. Sinds 1988 hebben wij dit terughoudende beleid gevoerd, waarbij we het gebruik van immunosuppressiva hebben beperkt tot die patiënten bij wie de nierfunctie duidelijk achteruit gaat. Daarnaast hebben wij gezocht naar risico factoren die het mogelijk maken patiënten 'at risk' voor progressieve ziekte in een vroege fase van de ziekte te identificeren. Ook hebben wij onderzoek gedaan naar betere immunosuppressieve medicijnen. In Figuur 1 geven we een overzicht van het verrichte onderzoek met verwijzing naar de relevante hoofdstukken.

We hebben eerst de effectiviteit geëvalueerd van immunosuppressieve behandeling van patiënten die het hoogste risico lopen op eindstadium nierfalen: patiënten met een duidelijke achteruitgang van nierfunctie (serum creatinine > 135 μmol/l en/of een stijging van het serum creatinine met > 50%). In ***Hoofdstuk 2*** rapporteren we de prospectieve studie bij 65 patiënten met idiopathische membraneuze glomerulopathie en nierinsufficiëntie, behandeld met cyclofosfamide en steroïden. Het behandelingsschema bestond uit oraal cyclofosfamide 1.5-2.0 mg/kg/dag gedurende 12 maanden en steroïden, methylprednisolon pulsen 3 x 1 g, i.v. op maand 0, 2 en 4 en oraal prednison 0.5 mg/kg om de dag gedurende 6 maanden. De mediane duur van follow-up was 51 maanden. Bij alle patiënten bleek de nierfunctie tenminste tijdelijk te verbeteren of te stabiliseren. Een partiële remissie (PR, proteïnurie van 0.21-2.0 g/10 mmol creatinine) trad op bij 56 patiënten; 17 patiënten verbeterden tot een complete remissie (CR, proteïnurie < 0.2 g/10 mmol creatinine). Gedurende de follow-up trad bij 11 patiënten een recidief (terugkeer) op van het nefrotisch syndroom (dit komt overeen met een recidiefpercentage van 28% na 5 jaar). Negen patiënten werden opnieuw behandeld met immunosuppressie wegens achteruitgang van nierfunctie. Aan het eind van de follow-up waren 16 patiënten in CR, 31 in PR, acht patiënten hadden een aanhoudend nefrotisch syndroom, een patiënt had milde proteïnurie, vier patiënten hadden eindstadium nierfalen ontwikkeld en vijf patiënten waren overleden. De renale overleving (levende patiënt zonder eindstadium nierfalen) was 86% na 5 jaar en 74% na 7 jaar.



Figuur 1. Rationele behandelings-strategie voor idiopatische membraneuze glomerulopathie

Om de effectiviteit van de immunosuppressieve therapie bij patiënten met achteruitgaande nierfunctie te illustreren, hebben we de renale overleving vergeleken van onze behandelde patiënten met die van een groep historische controle patiënten (*n*=24), die ofwel niet behandeld waren of behandeld waren met immunosuppressie waarvan we nu weten dat deze niet effectief is. De renale overleving in deze groep van historische controle patiënten was slechts 32% na 5 en 7 jaar.

Helaas ging de behandeling met immunosuppressiva gepaard met complicaties in tweederde van de patiënten. De bijwerkingen bestonden met name uit beenmergdepressie en infecties. Bovendien ontwikkelde een patiënt blaaskanker, een gevreesde complicatie van behandeling met cyclofosfamide.

Uit deze studie concluderen wij dat de renale overleving significant verbeterd kan worden door patiënten met idiopathische membraneuze glomerulopathie en nierinsufficiëntie te behandelen met cyclofosfamide en steroïden. Echter, er treden frequent bijwerkingen op en het aantal patiënten dat een recidief ontwikkelt neemt toe bij een langere follow-up.

In ***Hoofdstuk 3*** hebben we prospectief het klinische beloop bestudeerd in 15 patiënten, die ofwel niet hadden gereageerd op een eerste kuur met immunosuppressie dan wel een recidief van het nefrotisch syndroom hadden ontwikkeld gepaard gaande met achteruitgang van nierfunctie. Deze patiënten werden behandeld met een volgende kuur immunosuppressie. De eerste kuur immunosuppressie had bestaan uit chloorambucil of cyclofosfamide en was 8 maanden (spreiding: 0 - 143 maanden) na het verrichten van de nierbiopsie gestart. De tweede kuur bestond bij alle patiënten uit cyclofosfamide en steroïden. Het interval tussen de eerste en tweede kuur was 40 maanden (spreiding: 7 - 112 maanden). De totale follow-up was 110 maanden (spreiding: 46-289 maanden). Bij alle patiënten verbeterden de nierfunctie en de proteïnurie tenminste tijdelijk na de tweede kuur. Vier patiënten hadden nog een extra kuur nodig. Aan het eind van de follow-up waren twee patiënten in complete remissie, acht in partiële remissie, drie patiënten hadden aanhoudende proteïnurie, een patiënt was achteruitgegaan en ontwikkelde eindstadium nierfalen en een patiënt was overleden. De renale overleving kwam hiermee op 86% na 5 en 10 jaar follow-up. Het geven van herhaalde kuren immunosuppressie resulteerde in een winst in overlevingsduur zonder dialyse van meer dan 93 maanden (spreiding: 43-192 maanden). Concluderend denken wij dat een tweede kuur immunosuppressie geadviseerd zou moeten worden aan patiënten met een aanhoudend of recidiverend nefrotisch syndroom met nierinsufficiëntie.

Ondanks de effectiviteit van cyclofosfamide, noodzaakte het grote aantal bijwerkingen van de immunosuppressieve therapie ons tot het zoeken naar nieuwere behandelingsstrategieën, gericht op hoge effectiviteit met minder complicaties.

In ***Hoofdstuk 4*** rapporteren we de interim resultaten van een lopende pilot-studie, die gestart werd in mei 2002. In deze studie hebben we patiënten met nierinsufficiëntie behandeld met mycofenolaat mofetil (MMF), tweemaal daags 1000 mg gedurende 12 maanden in combinatie met steroïden volgens hetzelfde schema als hiervoor genoemd. Dertien patiënten hebben het behandelingsjaar afgerond en de resultaten van deze behandeling werden vergeleken met 13 gematchte historische controle patiënten die behandeld waren met cyclofosfamide. Het bleek dat MMF en cyclofosfamide even effectief waren in het verbeteren van nierfunctie en het verminderen van de proteïnurie. In het behandelingsjaar bereikten acht (MMF) respectievelijk zeven (cyclofosfamide) patiënten een partiële remissie van de proteïnurie. Ongeveer 70% van de patiënten had bijwerkingen bij beide behandelingsschema's. Patiënten die behandeld waren met cyclofosfamide hadden vooral last van leucopenie, terwijl de patiënten behandeld met mycofenolaat mofetil vooral leden aan anemie (bloedarmoede). In beide patiëntengroepen kwamen infecties voor. Hoewel aldus de frequentie van bijwerkingen niet minder hoog was, waren de bijwerkingen bij gebruik van mycofenolaat mofetil wel minder ernstig. Helaas ontwikkelden twee patiënten die behandeld waren met mycofenolaat mofetil een recidief nefrotisch syndroom nog tijdens of korte tijd na het staken van de behandeling. Derhalve moeten we de resultaten van behandeling van een grotere groep patiënten die langer vervolgd zijn afwachten, voordat we harde conclusies over de effectiviteit van mycofenolaat mofetil voor deze groep hoog-risico patiënten kunnen trekken.

In ***Hoofdstuk 5*** demonstreren we de voordelen van onze terughoudende behandelingsstrategie. Hiervoor hebben we prospectief een cohort van 69 patiënten bestudeerd, met een normale nierfunctie en een nefrotisch syndroom op het moment van de nierbiopsie. Immunosuppressieve therapie, die vooral bestond uit cyclofosfamide en steroïden, werd alleen geadviseerd aan patiënten met nierinsufficiëntie of een ernstig nefrotisch syndroom. Op het moment van de biopsie bedroeg het gemiddelde serum creatinine 90 µmol/l en de proteïnurie 6.7 g/dag. Na een follow-up van meer dan 5 jaar hadden 33 (48%) patiënten immunosuppressieve therapie gekregen, vooral wegens nierinsufficiëntie (n=24). Aan het eind van de follow-up waren 22 patiënten in complete remissie (32%), 24 patiënten in partiële remissie (35%), 15 patiënten hadden nog steeds een nefrotisch syndroom (22%), een

patiënt vertoonde milde proteïnurie (1.4%), zes patiënten hadden progressie vertoond tot eindstadium nierfalen (8.7%) en een patiënt was overleden. De renale overleving bedroeg hiermee 94% na 5 jaar en 88% na 7 jaar follow-up. Ter vergelijking, Ponticelli *et al.*, die 100% van de patiënten behandelden, rapporteerden een 10 jaars overlevingspercentage van 92%. Hieruit concluderen wij, dat een terughoudende behandelingsstrategie een gunstige prognose verzekert, terwijl in > 50% van de patiënten de blootstelling aan immuno-suppressieve therapie wordt voorkomen.

Niettemin kunnen we niet uitsluiten, dat de prognose nog verder verbeterd kan worden door patiënten die een hoog risico hebben op achteruitgang van hun nierfunctie in een vroegere fase te behandelen, dat wil zeggen voordat het serum creatinine significant begint te stijgen. Om dit te kunnen doen moeten we echter in staat zijn hoog-risico patiënten vroeg in het beloop van de ziekte te identificeren.

In ***Hoofdstuk 6*** hebben we de waarde gevalideerd van de uitscheiding in de urine van β_2-microglobuline en IgG, de eerder door ons gesuggereerde prognostische factoren. In een prospectieve studie, die in 1995 van start ging, hebben we bij 58 patiënten met een membraneuze glomerulopathie, normale nierfunctie en een nefrotisch syndroom, een gestandaardiseerde meting uitgevoerd ter bepaling van nierfunctie en de uitscheiding van laag- en hoog-moleculaire eiwitten in de urine. Het eindpunt 'nierdood' werd scherp gedefinieerd als een serum creatinine boven de 135 μmol/l (1.5 mg/dl), een stijging in het serum creatinine van > 50%, of de noodzaak tot het starten van immunosuppressieve behandeling. Na een follow-up tijd van meer dan 4 jaar, hadden 25 (43%) van de patiënten het eindpunt bereikt. In een multivariate analyse volgens het Cox proportional hazard model werd de β_2-microglobuline excretie in de urine als de sterkste onafhankelijke voorspeller voor de ontwikkeling van nierinsufficiëntie bevestigd, gebruikmakend van een grenswaarde van 0.5 μg/min, met een sensitiviteit van 83% en een specificiteit van 88%. De sensitiviteit en specificiteit waren resp. 84% en 85% voor de uitscheiding in de urine van IgG, bij toepassing van een grenswaarde van 250 mg/24 uur. Bij gebruik van de combinatie van beide eiwitten verbeterde de specificiteit naar 94%. Derhalve concluderen wij dat de uitscheiding in de urine van β_2-microglobuline en IgG bruikbare markers zijn en een leidraad kunnen vormen bij de beslissing omtrent het starten van immunosuppressieve therapie.

Helaas kan de uitscheiding in de urine van β_2-microglobuline alleen correct worden gemeten in alkalische urine. Om een urine pH boven de 6.0 te bewerkstelligen maken we gebruik van natriumbicarbonaat. Echter, in 7% van de gevallen wordt de pH-waarde van 6.0 niet bereikt. Wij onderzochten of het toedienen van acetazolamide, een carbo-anhydrase remmer, de urine voldoende zou kunnen alkaliniseren, zonder de uitscheiding in de urine van de diverse eiwitten te beïnvloeden. In ***Hoofdstuk 7*** laten we zien, dat de toepassing van acetazolamide de urine pH adequaat verhoogde, zodat het meten van de β_2-microglobuline excretie in de urine mogelijk bleek, zonder de hoogte van de excretie te beïnvloeden. Echter, er traden frequente bijwerkingen op, vooral bestaande uit paresthesiën (58%), smaakveranderingen (32%) en duizeligheid (29%). Hoewel deze bijwerkingen niet ernstig waren, belemmeren ze wel het routinematige gebruik van acetazolamide.

In het perspectief van de waarde van de uitscheiding in de urine van β_2-microglobuline en IgG ter voorspelling van het optreden van achteruitgang van nierfunctie bij patiënten met een normale nierfunctie, onderzochten we de veranderingen in de excretie van deze laag- en hoog-moleculaire eiwitten gedurende en na behandeling met immunosuppressie.
In ***Hoofdstuk 8*** beschrijven we het tijdsbeloop van de excretie in de urine van deze eiwitten na het starten van therapie en de waarde in het voorspellen van de uitkomst op de lange termijn. Elf patiënten ondergingen herhaalde gestandaardiseerde metingen van de uitscheiding in de urine van IgG, β_2-microglobuline en α_1-microglobuline tijdens het jaar van behandeling. We zagen een snelle verbetering in de selectiviteit van de glomerulaire permeabiliteit en van de tubulaire eiwitresorptie in de eerste twee maanden na het starten van de therapie. Echter, ondanks het optreden van een partiële remissie van de proteïnurie in de eerste 12 maanden bij de meeste patiënten, bleven er duidelijke tekenen van tubulo-interstitiële schade bestaan. Teneinde de predictieve waarde van de uitscheiding in de urine van de hoog- en laag-moleculaire eiwitten aan het eind van het behandelingsjaar te kunnen onderzoeken, werden bij 25 patiënten metingen verricht op 12 maanden. Het bleek echter, dat de urinewaarden voor IgG, β_2-microglobuline en α_1-microglobuline noch aan het begin van de behandeling noch op 12 maanden het optreden van een remissie of een recidief nefrotisch syndroom konden voorspellen. Zeventien patiënten ondergingen metingen 2-5 jaar na het starten van de immunosuppressieve behandeling. In het geval van een persisterende stabiele remissie zagen we een geleidelijke afname van het urine β_2-microglobuline tot normale waarden, terwijl in geval van een recidief nefrotisch syndroom het β_2-microglobuline in de urine steeg. De

verkregen waarden bij deze studie stelden ons in staat de relatie te onderzoeken tussen de tubulaire resorptie van β_2-microglobuline en de uitscheiding in de urine van IgG. Hieruit konden we concluderen dat de uitscheiding in de urine van β_2-microglobuline niet het gevolg is van het remmen van de resorptie ervan door IgG, waarmee de waarde van het β_2-microglobuline als marker voor tubulo-interstitiële schade werd bevestigd.

In ***Hoofdstuk 9*** stellen we een rationele behandelingsstrategie voor ter behandeling van patiënten met idiopathische membraneuze glomerulopathie, gebaseerd op een evaluatie van de literatuurgegevens hieromtrent en onze eigen ervaringen. De voorgestelde strategie wordt weergegeven in Figuur 1.

Dankwoord

Dankzij de inspiratie, hulp en steun van velen heb ik mijn onderzoek tot een goed einde kunnen brengen. Graag wil ik een ieder hiervoor hartelijk danken, ook degenen die ik niet bij naam noem.

Om te beginnen wil ik natuurlijk de vele patiënten bedanken, die veelal van heinde en verre voor een zogenaamde gestandaardiseerde urinemeting en behandelingsadvies naar de afdeling nefrologie van het Universitair Medisch Centrum St. Radboud te Nijmegen kwamen. Zij werden verwezen door welwillende collega nefrologen, uit aanvankelijk vooral Zuid-Oost Nederland, die onze behandelingsadviezen opvolgden, patiënten waar mogelijk in studies includeerden en ons in de gelegenheid stelden het beloop van de patiënten te vervolgen. Zonder de medewerking van deze patiënten en collegae had dit proefschrift niet tot stand kunnen komen. Mijn dank hiervoor!
Ik hoop dat de groeiende samenwerking tussen de nefrologen in Nederland zal leiden tot meer onderzoek en behandelingen in onderzoeksverband om zo samen wijzer te worden en patiënten zo optimaal mogelijk te kunnen behandelen.

Natuurlijk gaat mijn hartelijke dank uit naar de stafleden en nefrologen in opleiding van de afdeling nierziekten van het Universitair Medisch Centrum St. Radboud. Zij hebben niet alleen bijgedragen aan een gedegen opleiding tot nefroloog en een stimulerend onderzoeksklimaat, maar waren zelfs bereid als proefkonijn te dienen bij het acetazolamide onderzoek.

Bedankt ook Annemiek Wellesen-Polman en Simone Mooren, research-verpleegkundigen. Zij verrichten niet alleen talloze urinemetingen en registreerden de resultaten in de database, maar gaven ook anderszins de nodige ondersteuning.

Ina Klasen, Gertrude van de Wiel en de overige medewerkers van het klinische chemisch laboratorium wil ik danken voor hun bijdrage aan de urinemetingen en vooral het acetazolamide onderzoek.

Amanda Branten, heel hartelijk dank voor al je hulp en gezelligheid, waardoor de Nijmeegse maandagen, zoals ik ze in de laatste jaren heb gekend, niet alleen efficiënt maar ook gezellig waren. Samen verzamelden we op locatie patiëntengegevens en worstelden we met de queries in Access. Je hielp me op gang met de statistische bewerkingen en het maken van figuren. Maar vooral ook heel hartelijk dank voor je uitvoerige hulp in de laatste fase op allerlei gebied. Laten we het samen tot een goed einde brengen.

De grootste bijdrage aan dit onderzoek werd zonder meer geleverd door mijn promotor, Prof. Dr. J.F.M. Wetzels, als groot initiator en stimulator. Jack, jij wist me over te halen toch aan wetenschappelijk onderzoek te beginnen en het ook nog af te ronden ondanks mijn vestiging in de periferie. Ik heb bewondering voor je inzicht en genoten van de discussies. Hoewel ik je rode pen menigmaal heb vervloekt, waardeer ik je schrijftalent en je vermogen om resultaten van studies zo beknopt en helder mogelijk weer te geven. Ook je altijd snelle respons, ondanks je eigen drukke leven, heb ik zeer gewaardeerd. Daarnaast ben je niet alleen een goede onderzoeker, maar ook een uitstekende clinicus. Ik heb veel van je geleerd en prijs me gelukkig ook nu nog vanuit het Bredase gebruik te mogen maken van je kennis. Tot slot, Jack en Amanda, bedankt voor jullie medeleven en steun in de moeilijke afgelopen maanden.

Marika van Leeuwen-Artz, dankzij onze geslaagde duobaan heb ook ik mijn onderzoek nog in een promotie kunnen laten eindigen. Het is prettig een klankbord te hebben voor mijn medisch handelen en te weten dat er bij mijn afwezigheid goed voor mijn patiënten wordt gezorgd. Ik hoop dat, nu we beiden gepromoveerd zijn, we tot rust komen om meer van het leven te kunnen genieten. Zou het een versnellinkje lager kunnen?

Beste familie en vrienden, bedankt voor jullie belangstelling en voor de aanmoediging tot het afronden van deze klus. Dat we het samen nog vaak gezellig mogen hebben.

Lieve pap, bedankt voor het bieden van de mogelijkheid te studeren en je stimulatie tot het opbouwen van een eigen carrière. Ik heb je tempo en doorzettingsvermogen mogen erven. Wat zou je trots geweest zijn, als je deze gelegenheid nog had mogen meemaken.

Lieve mam, jij bent van onschatbare waarde in ons gezin. Jouw onbegrensde inzet en liefdevolle opvang van ons gezin en in het bijzonder onze kinderen heeft gemaakt dat ik mijn handen vrij had voor mijn werk en onderzoek. Hier zijn geen woorden voor. Ik hoop dat mijn kinderen later op een net zo'n fijne jeugd kunnen terugkijken als ik. Bedankt, mam.

De meeste dank ben ik echter verschuldigd aan mijn echtgenoot. Lieve Gerard, jij hebt vooral in het begin een zeer belangrijke bijdrage aan het proteïnurie-onderzoek in zijn geheel geleverd door het schrijven van een Access database, waar we alle tot dan toe beschikbare data in de vorm van losse Excel bestanden konden inlezen. De daarop volgende jaren zijn hieraan talloze patiëntengegevens toegevoegd, heb je de database steeds weer aan onze wensen aangepast en hielp je ons op weg bij het extraheren van gegevens middels queries. Er bestaat nu een zeer waardevolle, lopende database, waar nog menig onderzoeker gebruik van zal kunnen maken. Daarnaast heb je belangrijk bijgedragen aan de lay-out van dit boekje.
Maar naast deze letterlijk technische ondersteuning, heb je vooral een grote bijdrage geleverd in het bieden van de ruimte voor het investeren in mijn werk en onderzoek. Jouw ongekende relativeringsvermogen, positivisme en vertrouwen in mij heeft een belangrijke bijdrage geleverd aan mijn carrière en het afronden van dit boekje.
Lieve Koen en Vera, jullie liefde en vrolijkheid doen mij ieder dag beseffen dat het leven een prachtig geschenk is.
Lieve Gerard, Koen en Vera, ik hoop dat we samen nog lang kunnen genieten.

Publicaties

1. van Puijenbroek EP, ***du Buf-Vereijken PWG***, Spooren PF, van Doormaal JJ. Possible increased risk of rhabdomyolysis during concomitant use of simvastatin and gemfibrozil. *J Intern Med* 1996; 240: 403-404
2. ***du Buf-Vereijken PWG***, Netten PM, Wollersheim H, Festen J, Thien T. Skin vasomotor reflexes during inspiratory gasp: standardization by spirometric control does not improve reproducibility. *Int J Microcirc Clin Exp* 1997; 17: 86-92
3. ***du Buf-Vereijken PWG***, Hilbrands LB, Wetzels JF. Partial renal vein thrombosis in a kidney transplant: management by streptokinase and heparin. *Nephrol Dial Transplant* 1998; 13: 499-502
4. ***du Buf-Vereijken PWG***, van der Ven AJ, Meis JF, Lemmens JA, van der Meer JW. Swelling of hand and forearm caused by Mycobacterium bovis. *Neth J Med* 1999; 54: 70-72
5. ***du Buf-Vereijken PWG***, van der Ven AJ, Meis JF, Lemmens JA, van der Meer JW. Reply to letter to the editor of J.H. van Loenhout-Rooyackers [published in the September issue of The Netherlands Journal of Medicine (1999;55:163)]. *Neth J Med* 1999; 55: 249
6. ***du Buf-Vereijken PWG***, Branten AJ, Wetzels JF. Cytotoxic therapy for membranous nephropathy and renal insufficiency: improved renal survival but high relapse rate. *Nephrol Dial Transplant* 2004; 19: 1142-1148
7. ***du Buf-Vereijken PWG***, Feith GW, Hollander D, *et al*. Restrictive use of immunosuppressive treatment in patients with idiopathic membranous nephropathy: high renal survival in a large patient cohort. *Q J Med* 2004; 97: 353-360
8. ***du Buf-Vereijken PWG***, Wetzels JF. Efficacy of a second course of immuno-suppressive therapy in patients with membranous nephropathy and persistent or relapsing disease activity. *Nephrol Dial Transplant* 2004; 19: 2036-2043
9. Branten AJ, ***du Buf-Vereijken PWG***, Klasen IS, *et al*. Urinary excretion of β_2-microglobulin and IgG predict prognosis in idiopathic membranous nephropathy: A validation study. *J Am Soc Nephrol* 2005; 16: 169-174
10. ***du Buf-Vereijken PWG***, Wetzels JF. Treatment related changes in urinary excretion of high and low molecular weight proteins in patients with idiopathic membranous nephropathy and renal insufficiency. *Nephrol Dial Transplant* 2005; in press
11. ***du Buf-Vereijken PWG***, Branten AJ, Wetzels JF. Idiopathic membranous nephropathy: Outline and rationale of a treatment strategy. *Am J Kidney Dis* 2005; in press

Curriculum Vitae

Peggy du Buf-Vereijken werd op 28 april 1967 geboren te Weert. Na afronding van het ongedeeld VWO aan de Philips van Horne Scholengemeenschap in Weert startte zij in 1985 met haar studie geneeskunde aan de Radboud Universiteit Nijmegen. Zij behaalde zowel haar propedeuse (1986) als haar artsexamen (1992) cum laude. Tijdens haar co-schappen liep zij een keuze co-schap tropengeneeskunde in het Sumve Districts Hospital, Sumve, Tanzania (1992), wat niet alleen een leerzame tijd was, maar vooral een bijzondere levenservaring.
Na haar artsexamen werkte zij als arts-assistent op de afdeling nucleaire geneeskunde van het Universitair Medisch Centrum St. Radboud te Nijmegen (hoofd Prof. Dr. F.H.M. Corstens). Op 1 juli 1993 startte zij met de opleiding tot internist in het TweeSteden Ziekenhuis, locatie Tilburg (toenmalige Maria Ziekenhuis; opleider Dr. L.G. van Doorn). Van eind 1994 tot juli 1999 vervolgde zij deze opleiding in het Universitair Medisch Centrum St. Radboud te Nijmegen (opleider Prof. Dr. J.H.M. van der Meer). In april 1999 werd aangevangen met de aantekening nefrologie (opleiders Prof. Dr. R.A.P. Koene en Prof. Dr. J.H.M. Berden), welke zij in oktober 2001 afrondde. Tijdens de laatste fase van de specialisatie tot nefroloog startte zijn met het wetenschappelijk onderzoek betreffende de prognose en behandeling van patiënten met een membraneuze glomerulopathie onder de inspirerende leiding van Prof. Dr. J.F.M. Wetzels. Op 1 mei 2003 trad zij toe als internist-nefroloog tot de maatschap interne geneeskunde en maag-darm-leverziekten van het Amphia Ziekenhuis Breda. Naast haar klinische werkzaamheden aldaar continueerde zij het wetenschappelijk onderzoek, uitmondend in dit proefschrift.
Zij is getrouwd met Gerard en de trotse moeder van Koen (1998) en Vera (2000).