Idiopathic Membranous Nephropathy: Diagnosis and Treatment

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Idiopathic membranous nephropathy is still the most common glomerular disease associated with nephrotic syndrome. The greater the proteinuria, the greater the long-term risk for renal failure. In addition, patients who have membranous nephropathy with nephrotic syndrome have significant morbidity and mortality, in particular related to thromboembolic and cardiovascular complications. There is no specific treatment for membranous nephropathy. Supportive care with the use of diuretics and angiotensin-converting enzyme inhibitors in combination with angiotensin II receptor blocker is recommended, but these agents have only a limited effect. Immunosuppressive treatment options include the use of corticosteroids, alkylating agents, cyclosporin A, tacrolimus, and mycophenolate mofetil, but their use is controversial, not all have been shown to be effective, and their use can be associated with significant adverse effects. This has resulted in relatively small improvement in the prognosis of membranous nephropathy in the past 30 yr, with up to 40% of patients eventually reaching end-stage renal failure. Agents that offer more complete response rates with lower adverse effects are truly needed. Recent data suggest that B cells play a key role in the pathogenesis of a number of autoimmune diseases including membranous nephropathy and that selective depletion of B cells in humans may be beneficial in preventing the production of pathogenic immunoglobulins and subsequent renal injury.

Case Presentation

A 32-yr-old man underwent a general medical examination at his primary care physician’s office in February 2004. At that time, he was noted to have elevated total cholesterol of 353 mg/dl and triglycerides of 417 mg/dl and was started on simvastatin 40 mg/d. During the course of the next 2 mo, he started developing swelling in his feet and ankles that proceeded to involve his calves. He also developed tenderness on his left calf. A diagnosis of a deep venous thrombosis was made, and he was started on oral anticoagulant therapy. Given the increased lower extremity edema, a urinalysis was conducted, and the patient was found to have significant proteinuria, with a 24-h urine collection showing proteinuria of 9.3 g. The patient had no history of diabetes, macroscopic hematuria, or hypertension. The patient underwent extensive serologic workup including monoclonal protein studies, serum complement levels, anti-neutrophil cytoplasmic antibodies, hepatitis B and C and HIV serology, and anti-nuclear antibody, all of which were negative or in the normal range, and in July 2004, he was referred to our institution for further evaluation. At presentation at the Mayo Clinic, the physical examination was of a healthy-appearing young man. His BP was 136/87 mmHg, pulse rate was 69, weight was 89.8 kg, and body mass index was 29 kg/m². Eyes, nose, and throat examination was normal. There was no lymphadenopathy. Heart examination showed regular rate and rhythm with no murmurs. Lungs were clear to auscultation. Abdomen was soft, with no masses or organomegaly. Lower extremities revealed 2+ pitting edema to the knees. Laboratory evaluation showed hemoglobin of 15.1 g/dl, leukocytes of 4.7 × 10⁹/L, and platelets of 236 × 10⁹/L. Serum creatinine was 1 mg/dl, serum albumin was 2.5 g/dl, total cholesterol was 260 mg/dl, and serum triglycerides were 224 mg/dl. Proteinuria was 8.6 g/24 h, and creatinine clearance (CrCl) was 110 ml/min. Chest x-ray was normal. Renal ultrasound showed a left kidney measuring 12.7 cm and a right kidney measuring 12.6 cm in length, with normal echotexture and negative for hydronephrosis or masses. Renal veins were patent bilaterally. The patient was started on a low-sodium (<4 g/d) and low-protein (0.8 g/kg per d high-quality protein) diet, simvastatin was changed to atorvastatin (20 mg/d), and he was initiated on lisinopril 20 mg/d. It was also recommended that he undergo a kidney biopsy to delineate further the diagnosis. This was performed 1 mo later. A week before the biopsy, oral anticoagulation therapy was discontinued. It was restarted 2 d after the renal biopsy was performed.

Dr. Fernando Fervenza: I have invited Dr. Sanjeev Sethi to discuss the renal biopsy findings.

Dr. Sanjeev Sethi: The renal biopsy consisted of one core of renal cortex, which contained 11 glomeruli. None of the glomeruli were globally sclerosed. One glomerulus showed segmental sclerosis with adhesion of the sclerosed segments to the Bowman’s capsule (Figure 1A). The glomeruli showed slightly thickened glomerular basement membranes (GBM), and on...
silver stains fine subepithelial pinholes were noted (Figure 1B). Basement membrane spikes were not evident. The glomerular did not show proliferative features, and there was no evidence of crescents, fibrinoid necrosis, or endocapillary proliferation. The mesangium was unremarkable with no significant increase in mesangial matrix or cellularity. The interstitium was well preserved, and significant tubular atrophy and interstitial fibrosis were not seen. Arteries were unremarkable, while arterioles showed mild focal hyaline deposition. Immunofluorescence microscopy showed granular staining for IgG (3+/3+; Figure 1C), C3 (2+/3+; Figure 1D), κ light chains (3+/3+), and λ light chains (3+/3+) along the capillary walls. Stains for IgA, IgM, and C1q were negative. Electron microscopy (EM) showed numerous scattered subepithelial deposits; some of the deposits were separated from each other by basement membrane spikes (Figure 1E). Intramembranous and subendothelial deposits were not present. There was extensive effacement of the foot processes of the visceral epithelial cells. Endothelial cells were unremarkable and did not contain tubuloreticular inclusions. The mesangium was also unremarkable and did not contain electron-dense deposits. Diagnosis: Membranous nephropathy (MN) with secondary focal segmental glomerulosclerosis (FSGS).

Discussion

The diagnosis of MN is based on the following findings: (1) Thickened GBM, often showing pinholes and spikes on silver and periodic acid-Schiff stains, and occasionally subepithelial fuchsinophilic deposits on trichrome stains; (2) immunofluorescence microscopy showing granular Ig, usually IgG and C3, along the capillary walls; and (3) subepithelial deposits on EM.

Based on the location of the deposits on EM, MN has been divided into four stages: Stage I, sparse small deposits without thickening of the GBM; stage 2, more extensive subepithelial deposits with formation of basement membrane spikes between the deposits and thickening of the GBM; stage 3, combination of stage 2 along with deposits completely surrounded by basement membrane (intramembranous deposits); and stage 4, incorporation of deposits in the GBM and irregular thickening of the GBM. From the pathology standpoint, it is important to determine whether the MN may be due to a secondary cause such as an autoimmune disease, neoplasia, infection, or drugs. Although it is often difficult to determine whether the MN is idiopathic or secondary, there are some features that are helpful in identifying a secondary cause. Features in favor of a secondary cause, in particular an autoimmune disease, include (1) proliferative features (mesangial or endocapillary), (2) full-house pattern of Ig staining including staining for C1q on immunofluorescence microscopy, (3) glomerular deposits predominantly containing Ig other than IgG4, (4) electron-dense deposits in the subendothelial location of the capillary wall and mesangium or along the tubular basement membrane and vessel walls, and (5) endothelial tubuloreticular inclusions on EM (1). EM showing only a few superficial scattered subepithelial deposits may suggest a drug-associated secondary MN.

Histologic findings such as the presence of FSGS, interstitial foam cells, extent of tubulointerstitial fibrosis, and vascular sclerosis have been associated with increased risk for renal failure (2–6), but whether they are independent of the clinical variables at the time of biopsy, can predict the rate of disease progression, or should guide therapy is uncertain. The EM location of the subepithelial deposits (i.e., subepithelial [homo- geneous, synchronous] with subgroups superficial and deep versus subepithelial and intramembranous [heterogeneous, multi-stage] deposits) has also been suggested as helpful in identifying patients who are most likely to respond to treatment, with patients in the homogeneous group showing a better long-term prognosis than patients in the heterogeneous group (7). On the other hand, a recent review of 389 renal biopsies of adult patients with MN showed that although the degree of tubulointerstitial disease, vascular sclerosis, and secondary FSGS were associated with reduced renal survival, they did not predict this outcome independent of the baseline clinical variable or correlate with the rate of decline in function or with baseline proteinuria (8). Furthermore, the severity of tubulointerstitial and vascular lesions did not preclude a remission in proteinuria in those who received immunosuppressive therapy. Similarly, neither the stage nor the synchronicity of the
Primary glomerular diseases such as IgA nephropathy and areas and especially in black individuals (9). In contrast to other most common cause of NS in white adults in many geographic Clinical Discussion was ezetimibe 10 mg/d. His CrCl was 117 ml/min, and proteinuria was increased to 40 mg/d. A month later, BP was 115/67 mmHg, but proteinuria had increased to 9.7 g/24 h. Atorvastatin dose was increased to 40 mg/d, and losartan at a dosage of 100 mg/d was added to his treatment. Attempts to increase lisinopril further resulted in postural hypotension. In December 2004, the patient returned for follow-up. BP was 107/58 mmHg, and his pulse rate was 69. Despite excellent controlled BP and his CrCl was 117 ml/min, and proteinuria was <0.2 g/24 h (Figure 2).

Clinical Discussion

Idiopathic MN is said to be the leading cause of nephrotic syndrome (NS) in white adults, although recent data, including our own, suggest that FSGS may have overtaken MN as the most common cause of NS in white adults in many geographic areas and especially in black individuals (9). In contrast to other primary glomerular diseases such as IgA nephropathy and idiopathic membranous nephropathy. FSGS was noted in one glomerulus (of 11). Other pertinent findings with good prognostic findings were that there was no global glomerulosclerosis, the interstitium was well preserved, no interstitial foam cells were noted, significant vascular sclerosis was not present, and finally EM showed superficial subepithelial deposits in a homogenous pattern.

In the patient discussed, were there no features to suggest a secondary cause of the membranous glomerulopathy. FSGS was noted in one glomerulus (of 11). Other pertinent findings with good prognostic findings were that there was no global glomerulosclerosis, the interstitium was well preserved, no interstitial foam cells were noted, significant vascular sclerosis was not present, and finally EM showed superficial subepithelial deposits in a homogenous pattern.

Dr. Fernando Fervenza: The subsequent clinical course was as follows. Secondary causes of MN were ruled out; namely, there was no history of nonsteroidal anti-inflammatory agent usage, hepatitis B and C serology were negative, and antinuclear and anti–double-stranded DNA antibodies testing was also negative. The patient was recommended to undergo conservative treatment aiming at maximizing renal protection therapy and minimizing proteinuria, and lisinopril dosage was increased to 40 mg/d. A month later, BP was 115/67 mmHg, but proteinuria had increased to 9.7 g/24 h. Atorvastatin dose was increased to 40 mg/d, and losartan at a dosage of 100 mg/d was added to his treatment. Attempts to increase lisinopril further resulted in postural hypotension. In December 2004, the patient returned for follow-up. BP was 107/58 mmHg, and his pulse rate was 69. Despite excellent controlled BP and proteinuria increased to 10.8 g/24 h. An extensive discussion regarding alternative options besides maintaining the status quo was carried on with the patient, including the use of cyclosporine (CsA), tacrolimus (TAC), corticosteroids in combination with a cytotoxic agent, and mycophenolate mofetil (MMF). He was also informed of our clinical study on the use of rituximab in MN. After having thought it over and reviewing the informed consent form, the patient decided to participate in the rituximab trial. As per protocol, the patient received two intravenous infusion of rituximab at a dosage of 1000 mg on days 1 and 15. After rituximab infusions, proteinuria decreased to 5, 1.7, 1, and 0.2 g at 1, 3, 6, and 12 mo, respectively. Since then, the patient has remained in complete remission of proteinuria. At his last follow-up in June 2007, his BP was 102/65 mmHg while taking lisinopril 40 mg/d and his CrCl was 117 ml/min, and proteinuria was <0.2 g/24 h (Figure 2).

Clinical Manifestations

The disease affects patients of all ages and races but is more common in men than in women and most often diagnosed in middle age with the peak incidence during the fourth and fifth decades of life (12). At presentation, the great majority of patients are normotensive, with hypertension present in 10 to 20% of the cases and severe hypertension in 5 to 10% (12). Renal function may be normal at diagnosis or may be abnormal at diagnosis, with GFR ranging from 20 to 100 ml/min/1.73 m², and with a normal or slightly increased serum creatinine (20 to 25%) having significant renal insufficiency (12,13).

Natural History

MN is a chronic disease, with spontaneous remission and relapses. Spontaneous remissions are said to occur in up to 30% of patients (approximately 60%) who present with subnephrotic-range proteinuria at the time of diagnosis (12). I would also like to emphasize that a significant number of patients (approximately 60%) who present with subnephrotic-range proteinuria will progress to full NS within 1 to 2 yr from presentation (Daniel C. Cattran, University of Toronto, Canada, unpublished observations, 2006). The presence of microscopic hematuria is common (30 to 40%), but macroscopic hematuria and red cell casts are rare and suggest a different diagnosis (13). At presentation, the great majority of patients are normotensive, with hypertension present in 10 to 20% of the cases and only a small fraction of patients (<20%) having significant renal insufficiency (12,13).

Figure 2. Course of proteinuria after two doses of rituximab (RTX; 1 g each). Note that proteinuria continues to increase despite maximally tolerated doses of angiotensin-converting enzyme inhibitor (ACEi) used in combination with an angiotensin receptor blocker (ARB).
of cases and usually occur within the first 2 yr after presenta-
tion. The percentage of patients going into spontaneous remis-
sion is, however, much lower when patients are selected with 
higher grades of proteinuria at presentation (e.g., proteinuria 
>8 g/24 h). The other two thirds of the patients will generally 
divide equally into either those with persistent proteinuria who 
will maintain renal function long term or patients who will 
progress to renal failure. In white patients with NS, 10-yr 
kidney survival of >70% has been reported (3,14–17). In my 
opinion, however, these data need to be carefully considered 
because many of these studies include a significant number of 
patients with proteinuria <3.5 g/24 h. For example, Schieppati 
et al. (16) reported a 72% renal survival at 8 yr for 100 
untreated patients with MN; however, in this study, 37% of patients 
were non-nephrotic (56% of patients had proteinuria <5 g/24 h), 
the median follow-up was only 39 mo, and deaths were excluded 
from the analysis. Despite these exceptions and the “benign” 
presentation characteristics, 25% of the patients reached ESRD 
by the end of 8 yr (16). Thus, it should not come as a surprise 
that both in the United States and Europe, MN remains the 
second or third leading cause of ESRD among the primary 
glomerulonephritis types (18). Even patients who do not 
progress but remain nephrotic are at an increased risk for 
life-threatening thromboembolic and cardiovascular events. A 
rapid change in either the degree of proteinuria or the rate of 
loss of renal function, especially in a previously stable patient, 
should raise the possibility of a superimposed condition (e.g., 
acute renal vein thrombosis, superimposed crescentic glomer-
ulonephritis, anti-GBM disease).

Predicting Factors
Evaluating the prognosis is critical in making the decision 
regarding when and what to use in terms of treatment (e.g., 
conservative versus immunosuppressive treatment in patients 
with MN) (5,19,20). An accurate predictor of outcome of pa-
tients with MN would allow the separation of patients who are 
likely to have long-term renal survival from those who are 
likely to progress. This would allow us to target immunosuppres-
sive treatment to patients at high-risk for renal disease 
progression; however, finding useful markers that predict this 
last group has been difficult. As discussed by Dr. Sethi earlier, 
the current evidence does not support that pathology plays 
much of a role other than diagnosis: It is not helpful in estab-
lishing prognosis or predicting response to immunosuppres-
sive therapy. Urinary excretion ratios of α1-microglobulin, β2-
microglobulin, IgM, and IgG have all been found helpful in 
assessing the severity of the overall renal injury and to predict 
outcome in MN (21–25). Unfortunately, quantification of uri-
nary α1-microglobulin, β2-microglobulin, IgM, and IgG is not 
widely available, and this limits their clinical use. Thus far, 
the best model for the identification of patients at risk was de-
veloped with data derived from the Toronto Glomerulonephritis 
Registry (26,27). This model takes into consideration the initial 
CrCl, the slope of the CrCl, and the lowest level of proteinuria 
during a 6-mo observation period. This risk score assessment 
has good performance characteristics and has been validated in 
two geographically diverse MN populations, one from Italy, 
the other from Finland (27). Based on this model, patients who 
present with a normal CrCl, proteinuria <4 g/24 h, and stable 
renal function over a 6-mo observation period have an excellent 
long-term prognosis and are classified as at low risk for pro-
gression. Patients with normal renal function and whose CrCl 
remains unchanged during 6 mo of observation but continue to 
have proteinuria >4 g but <8 g/24 h have a 55% probability of 
developing chronic renal insufficiency and are classified as 
medium risk for progression, and patients with persistent pro-
teinuria >8 g/24 h, independent of the degree of renal dys-
function, have a 66 to 80% probability of progression to chronic 
renal failure within 10 yr and are classified in the high risk for 
progression category. Patients with MN who were never ne-
phrotic or who achieved a complete remission (CR) of protein-
uria have an excellent long-term renal survival. Even a partial 
remission (PR) has been recognized as a predictor of long-term 
positive outcome in patients with MN (28). Troyanov et al. (28) 
recently reviewed the data on 350 patients with MN and NS 
and found that the 10-yr renal survival was 100% in the CR 
group, 90% in the PR group, and 45% in the no remission 
group. Patients in CR or PR have a similar rate of decline: −1.5 
ml/min per yr in the CR group and −2 ml/min per yr in the 
PR group. In contrast, the no remission group lost GFR at a rate of 
−10 ml/min per yr. A review of this algorithm was recently 
published (19).

Treatment Options
Using the algorithm for predicting outcome described, we can 
rationally assign patients to conservative, nonimmunosuppres-
sive therapy or to immunosuppressive therapy according to 
their risk for renal disease progression.

Conservative Therapy
Conservative therapy consists of restricting dietary protein in-
take (0.8 g/kg ideal body wt per d high-quality protein) and 
controlling BP (target BP is ≤125/75 mmHg), hyperlipidemia, 
and edema. In the Modification of Diet in Renal Disease 
(MDRD) study, patients with proteinuria >1 g/d had a signif-
icantly better outcome when their BP was reduced to 125/75 
mmHg (29). Thus, in patients with proteinuric renal disease 
and including MN patients, the current target for BP control is 
≤125/75 mmHg. Reducing protein intake to approximately 0.6 
to 0.8 g/kg ideal body wt per d decreases nephrotic-range 
proteinuria (30); however, dietary protein restriction alone is 
unlikely to induce CR of the NS. ACEi and/or ARB are effective 
antihypertensive agents that can reduce proteinuria and slow 
progression of renal disease in both diabetic and nondiabetic 
chronic nephropathy patients, and for these reasons they are 
the preferred agents to treat hypertension in proteinuric renal 
diseases; however, controlled trials of ACEi or ARB in patients 
with MN have been few and far between. The evidence that 
such therapy is beneficial in MN is weak and largely inferential, 
and the following issues need to be considered when using an 
ACEi and or an ARB in patients with MN: (1) In both diabetic 
and nondiabetic nephropathy patients, the use of an ACEi 
and/or an ARB reduces proteinuria, slows progression of renal 
disease, and seems to be cardioprotective (31); however, the
degree of renal protection is related to the degree of proteinuria reduction, and if proteinuria is not lowered, then the benefit is substantially attenuated (32,33). The most recent data from the Reduction of Endpoints in NIDDM with the Angiotensin II Antagonist Losartan (RENAAL) trial confirm these observations in that the renoprotective effect of angiotensin II blockade in patients with diabetic nephropathy was nearly fully explained by its antiproteinuric effect (34). (2) In patients with MN, the antiproteinuric effect is modest (<30% decrease) and is more significant in patients with lower levels of proteinuria (35–37). (3) Thus, in contrast to diabetic renal diseases, ACEI may not offer the same degree of renal protection to patients with MN (38). In fact, studies by du Buf-Vereijken et al. (17) and in a review by Troyanov et al. (28), the use of ACEI or ARB by multivariate analysis did not show an independent value in determining the prognosis of patients with MN. Furthermore, Praga et al. (39,40) showed that in patients with NS (the majority with MN), ACEI were ineffective in reducing proteinuria and that this response in patients with MN was associated with a poor renal function outcome. (4) In patients in whom a significant antiproteinuric response is observed, the effect is usually seen within 2 mo of initiation of angiotensin II blockade therapy (35). Although a relative reduction of proteinuria is always a positive result, the aim of antiproteinuric therapy is to reduce it as close as possible to normal levels (CR). Reaching this goal in patients with proteinuria >5 g/24 h using conservative treatment with ACEI or ARB seems unrealistic, even when these drugs are used at the highest dosage. The case I presented today clearly emphasizes this point. Lipid-lowering medications (in particular the statins) may have a synergistic antiproteinuric effect when combined with ACEI, but this effect is small and mainly observed in patients with proteinuria <3 g/24 h (41,42); however the lipid abnormalities are important players in the high cardiovascular risk associated with proteinuria and thus are an important target for treatment in these patients.

For patients at low risk for progression (normal renal function and proteinuria <4 g/24 h), treatment with ACEI with or without ARB and a statin may be enough to reduce proteinuria to subnephrotic levels and is associated with little long-term significant adverse effects. Evidence to support this approach comes from published validation studies and from recent data on the clinical relevance of PR (27,28). Although a relative reduction of proteinuria is always a positive result, the aim of antiproteinuric therapy should be to reduce proteinuria as close as possible to normal levels (CR), as well as to preserve renal function long term. Reaching this goal in patients with proteinuria >5 g/24 h using conservative treatment with ACEI or ARB seems unrealistic, even when these drugs are used at the highest dosage, and this point was illustrated by the present case. Patients need to be instructed to follow a low-salt diet because a high intake (e.g., 200 mg of Na or 4.6 g sodium/d) can significantly impair the beneficial effects of angiotensin II blockade.

**Immunosuppressive Therapy**

Several treatment strategies, including a variety of immunosuppressive agents, have been shown to be at least partially successful in reducing proteinuria in MN (43). The available evidence is presented according to the risk group (e.g., medium, high) that the patients in the studies most closely represent, because the risk of treatment in the low-risk patient is likely to exceed the benefits. It should be recognized, however, that this evidence is based on a paucity of randomized, controlled clinical trials or derived from noncontrolled or nonrandomized studies, which are not on equal footing with randomized, controlled clinical trials.

**Medium Risk for Progression.**

**Corticosteroids.** A study conducted by the Toronto Glomerulonephritis Study Group in which patients were assigned to receive either a 6-mo course of prednisone given on alternate days or no specific treatment showed no significant benefit of corticosteroid treatment alone in either induction of a remission in proteinuria or preservation of renal function, even after the data were adjusted to include only patients with nephrotic-range proteinuria at entry (>3.5 g/24 h) (44).

**Combined Corticosteroids and Cytotoxic Agents.** A 6-mo course of methylprednisolone (MTP) intravenously and oral steroids alternating monthly with chlorambucil was superior to conservative treatment in achieving CR or PR (45). CR was achieved in 50% and PR in 31% of the cases compared with a CR in 7% and PR in 24% of the control patients. After 10 yr of follow-up, patients treated with combination therapy had a 92% probability of renal survival compared with 60% in the control group, and only 8% of treated patients versus 40% of untreated ones had reached ESRD (46). Similar results were seen when the same cyclic combination of corticosteroids and chlorambucil was compared with cyclic corticosteroids and oral cyclophosphamide (CYC) (47). CR or PR was observed in 82% of patients assigned to MTP and chlorambucil versus 93% of patients assigned to MTP and CYC (P = 0.116, nonsignificant). Treatment with CYC was associated with fewer side effects, but renal function was equally preserved in both groups for up to 3 yr. These observations have been recently confirmed by Jha et al. (48), who reported the 10-yr follow-up of a randomized, controlled trial on 93 patients allocated to receive either a 6-mo course of alternating prednisolone and CYC or supportive treatment alone. Of the 47 patients treated with immunosuppressive therapy, 34 achieved remission (15 CR and 19 PR), compared with 16 (5 CR and 11 PR) of 46 in the control group (P < 0.0001). The 10-yr dialysis-free survival was 89 and 65% (P = 0.016), and the likelihood of survival without death, dialysis, and doubling of serum creatinine was 79% in the treated versus 44% in the control group (P = 0.0006). The incidence of infections was similar in the two groups (48). Ponticelli et al. (49) also compared the effect of 6 mo of alternating monthly pulses of MTP plus oral steroids and chlorambucil cycled as described versus MTP pulses plus steroids alone and found at 3 yr 66% of the patients given steroids and chlorambucil versus 42% of patients given steroids alone were in remission, the difference being significant. At 4 yr, this difference was no longer statistically significant, although a seemingly large 20% difference favoring the combined treatment persisted, with renal function been better preserved in the chlorambucil-treated group. Thus, in a number of studies, both CYC and chlorambucil in combination with corticosteroids.
have been effective in the treatment of patients with IMN and preserved renal function with benefits maintained well beyond the 1-yr treatment period, although relapse rates approached 35% at 2 yr. The long-term adverse effects of these cytotoxic agents, in particular, effects on fertility as well as bladder carcinoma and myelodysplasia are the major drawbacks to the universal application of this form of therapy. A recent publication suggested that the risk for malignancy is not increased for patients treated with cumulative CYC doses \( \geq 36 \text{ g} \) but increases significantly in patients with cumulative CYC doses \( \geq 36 \text{ g} \) (50). In regards to the risk for gonadal toxicity, I am not aware that this issue has been formally addressed in patients with MN treated with CYC; however, based on studies in patients with lupus nephritis, the risk seems to be age related: CYC resulted in ovarian failure in 100% of women older than 30 yr, approximately 50% of those aged 20 to 30 yr, and 13% of patients younger than 20 yr (51). Similarly, the average cumulative dose needed to cause amenorrhea seems to decrease with age: Doses of 20.4 g in women in their 20s, 9.3 g in women in their 30s, and as little as 5.2 g for women in their 40s (52). Thus, the risks are probably low in a patient who receives one single course of CYC, but CYC has a cumulative toxicity, and they should be taken into consideration, especially in patients who relapse after treatment and may require a second course of therapy.

CsA. In relation to CsA, a single-blind, randomized, controlled study of 51 patients with steroid-resistant MN treated with low-dose prednisone plus CsA and compared with placebo plus prednisone showed that at the end of 26 wk of treatment, 75% of patients (21 of 28) in the CsA group versus only 22% of patients (five of 23) in the controls had achieved PR or CR \( (P < 0.001; \text{CR} = 2 \text{ in the CsA group versus 1 in the placebo group}) \) (53). Relapses occurred in approximately 40% of patients within 1 yr of discontinuation of CsA treatment, very similar to that seen with combined cytotoxic/corticosteroid regimens. Relapses should not be considered failure of therapy because reintroduction of CsA or its alternative (i.e., cytotoxic/corticosteroid regimen) is usually capable of inducing another remission. Data from the German Cyclosporine in NS Study Group suggest that prolonging CsA treatment (>1 yr) results in higher (34% CR at 1 yr) and more sustained rate of remissions (54). Taken together, these data suggest that CsA can induce a remission (CR or PR) of the NS in 50 to 60% of patients. It is important to emphasize that although reduction of proteinuria usually occurs within a few weeks, the majority of CR occurred after more than 6 mo of treatment. On the other hand, if after 3 to 4 mo of CsA therapy at adequate doses proteinuria is not significantly reduced, then it is unlikely that the therapy will be effective. Prolonged low-dose CsA (approximately 1.5 mg/kg per d) could be considered for long-term maintenance of patients with preserved renal function who achieve CR or PR but who relapse once CsA is discontinued, with little risk for nephrotoxicity (55).

TAC. An alternative to CsA is the recent study by Praga et al. (56) evaluating TAC monotherapy in MN. In that study, 25 patients with normal renal function (mean proteinuria approximately 8 g/24 h) received TAC (0.05 mg/kg per d) over 12 mo with a 6-mo taper, whereas 23 patients served as control. After 18 mo, the probability of remission was 94% in the TAC group but only 35% in the control group. Six patients in the control group and only one in the TAC group reached the secondary end point of a 50% increase in their serum creatinine (56). Unfortunately, almost half of the patients relapsed after TAC was withdrawn, and similar to patients treated with CsA, maintenance of remission may require prolonged use of TAC in low dose.

**High Risk for Progression.**

Corticosteroids. The UK Medical Research Council conducted a randomized, prospective, double-blind, controlled trial assessing the medium-term effect of an 8-wk course of high-dose prednisolone in 52 patients with preserved renal function and high-grade proteinuria (mean 10.8 ± 6/24 h) and compared it with 51 patients treated conservatively (57). At 36 mo, there was no significant difference regarding the degree of proteinuria between the control and the treatment groups, with renal function deteriorating equally in both groups, thus confirming both the high risk for progression associated with patients with this degree of proteinuria and the lack of benefit of corticosteroids.

Combined Corticosteroids and Cytotoxic Agents. In relation to cytotoxic agents combined with corticosteroids, there has not been a randomized, controlled trial of cytotoxic agents plus corticosteroids in this high-risk group, although a number of uncontrolled studies have been conducted (46,47,49,58–69). In patients with deteriorating renal function, the supporting data are much less compelling than the results obtained in patients at medium risk for progression; adverse effects, especially infections, are higher; and the likelihood of benefit is reduced in patients with severe renal failure (serum creatinine >3 mg/dl).

CsA. In relation to CsA, there has been only one controlled trial using CsA in patients with high-grade proteinuria and progressive renal failure. In this study, of the initial 64 mem- branous patients, the 17 patients who had a loss in CrCl of ≥8 ml/min during the 12-mo observation (phase 1) period were randomly assigned to either CsA treatment (nine patients) or placebo (eight patients) for 12 mo (phase 2) (70). At the time of initiation of treatment, their average CrCl was in the mid-50s and their average proteinuria was 11 g/24 h. After 12 mo, there was a significant reduction in proteinuria, and the rate of loss (slope) of renal function in the CsA group was reduced from −2.4 to −0.7 ml/min per mo, whereas in the placebo group, the change was insignificant, −2.2 to −2.1 ml/min per mo \( (P < 0.02) \). This improvement was sustained in approximately 50% of the patients for up to 2 yr after CsA was discontinued.

MMF. No controlled trials are available regarding MMF in MN. Miller et al. (71), in a pilot study, treated 16 patients with MN with 1.5 to 2.0 g/d MMF for a mean of 8 mo. These patients would be categorized as either medium or high risk for progression given the severity of their proteinuria and the fact that they had previously failed a variety of other immunosuppressive drugs. The results were modest: Six patients had a ≥50% reduction in their proteinuria, two had a minor reduction in proteinuria, four had no change, three were withdrawn because of significant adverse effects, and one stopped treatment on his
own. Mean serum creatinine was unchanged over the course of the study. In patients who responded, the lowest degree of proteinuria was reached within 6 mo, suggesting that patients who are likely to respond would do so in this time frame. This pilot study is somewhat difficult to interpret as negative or positive, given the setting of resistance to all other agents. Similarly, Choi et al. (72) retrospectively analyzed 17 patients with MN treated with MMF who were steroid dependent, steroid resistant, or steroid intolerant, some had also been treated with CsA and had been resistant or had a suboptimal response to CsA. Overall, treatment with MMF (0.5 to 1.0 g twice daily for a mean of 12 mo), which was combined with steroids in most patients, resulted in a 61% reduction of proteinuria (7.8 to 2.3 g/24 h; P = 0.001), with eight patients having a PR and two patients a CR. Renal function improved in three of six patients with renal failure. More recently, Branten et al. (73) reported on 32 patients with MN and renal insufficiency (serum creatinine >1.5 mg/dl) treated with MMF (1 g twice daily) for 12 mo and compared with results obtained on 32 patients from a historic control group treated for the same period of time with oral CYC (1.5 mg/kg per d). Both groups received high-dose steroid treatment (MTP intravenously 1 g × 3 at months 1, 3, and 5 followed by oral prednisone 0.5 mg/kg every other day for 6 mo, with subsequent tapering). Overall, 21 MMF-treated patients developed PR of proteinuria; in six patients, proteinuria decreased by at least 50%; and no response was observed in five patients. Cumulative incidences of remission of proteinuria at 12 mo were 66% in the MMF group versus 72% in the CYC group (P = 0.3). Adverse effects occurred at a similar rate between the two groups, but relapses were much more common in the MMF-treated group (73).

I need to point out that the majority of the these studies were said to be targeted to patients with deteriorating renal function and have been deemed successful because proteinuria and azotemia were diminished. However, careful review of the data shows that reversal of azotemia is almost always incomplete and often transient, suggesting that the decline in renal function is merely attenuated and not arrested.

New Therapies

Adrenocorticotropic Hormone. In a study by Berg and colleagues (74,75), synthetic adrenocorticotropic hormone (ACTH) administered for 1 yr decreased proteinuria in patients with MN. More recently, Ponticelli et al. (76) conducted a randomized pilot study comparing MTP plus a cytotoxic agent versus synthetic ACTH in 32 patients with MN. Of these, 16 were randomly assigned to receive MTP plus chlorambucil or CYC (group A), and 16 were assigned to receive ACTH (group B). ACTH was administered by one intramuscular injection (1 mg) every other week with the frequency increased to two injections per week for a total treatment period of 1 yr. Data were reported according to intention-to-treat analysis. CR or PR as a first event was attained by 93% of patients in group A (five CR and 10 PR) and 87% in group B (10 CR and four PR), the difference between the two groups being nonsignificant. Adverse effects associated with the use of ACTH included dizziness, glucose intolerance, diarrhea, and the development of bronze-colored skin, which resolved after the end of therapy. Although these studies suggest that prolonged synthetic ACTH therapy may represent an effective therapy in patients with MN, more extensive randomized studies with longer follow-up are needed before therapeutic recommendations could be made. At the present time, the synthetic formulation of ACTH used in these studies is not available in the United States.

Rituximab. Experimental data in MN suggest that B cell activation results in Ig deposition along the GBM, causing injury to the membrane and subsequent proteinuria (77). In humans, there is evidence that therapy directed against B cells (e.g., cyclophosphamide, which has striking but nonselective effects on B cell function) is effective in MN (46,78). Thus, a case could be made for selectively targeting B cells and therefore halting the production of the pathogenic antibodies, resulting in improvement or even resolution of the glomerular pathology in MN. This form of therapy is available with the use of rituximab, a chimeric mAb directed against the cell-surface protein CD20, a molecule selectively expressed on cells of B lymphocyte lineage but not present on stem cells (79).

In a pilot study using rituximab in MN, Ruggenenti and colleagues (80,81) prospectively treated eight nephrotic patients with MN with four weekly courses of rituximab (375 mg/m²) and followed them for 1 yr. Proteinuria significantly decreased from a mean ± SD of 8.6 ± 4.2 g at baseline to 3.0 ± 2.5 g (−66%; P < 0.005) at 12 mo. This included two patients with <0.5 g/24 h and <3.5 g/24 h in three other patients. In the three remaining patients, proteinuria decreased by 74, 44, and 41%, respectively. Renal function remained stable in all patients. Adverse effects were reported as mild.

In collaboration with Dr. Daniel Cattran in Toronto, we recently conducted a prospective, open-label, pilot trial in 15 patients with MN and proteinuria >5 g/24 h despite treatment with ACEi and/or ARB and adequately controlled BP, with the present case being one of the patients enrolled in the study. Rituximab (1 g) was given on days 1 and 15. At 6 mo, patients with proteinuria >3 g/24 h and CD19+ B cell counts >15 cells/µl received a second identical course of rituximab. Thirteen men and two women, median age 47 (range 33 to 63) and mean ± SD baseline creatinine of 1.4 ± 0.5 mg/dl, were treated. Baseline proteinuria of 13.0 ± 5.7 g/24 h (range 6.1 to 23.5) decreased to 6.0 ± 7.3 g/24 h (range 0.2 to 20) at 12 mo (P < 0.001) (82). In the 14 patients who completed 12 mo of follow-up, CR (<0.3 g/24 h) was achieved in two patients, PR (<3 g/24 h) in six patients, and five patients did not respond. Two patients progressed to ESRD. Adverse effects were minor. The nearly 60% (eight of 14) CR or PR rate and a 48% reduction in mean protein levels are clinically important. Thus, rituximab seems effective in reducing proteinuria in some patients with MN, but this response varied from CR to no change, and our ability to identify a priori the patients who will respond to therapy remains elusive. A recent publication from Remuzzi’s group (83) suggested that rituximab is likely to be most effective in patients with minimal degrees of tubulointerstitial injury. Further research is needed to identify a priori which patients are likely to benefit from rituximab treatment. These pilot studies, although encouraging, need to be confirmed by ade-
quately powered, randomized, controlled studies before recommendations can be made regarding its use.

Dr. Fernando Fervenza:
I have invited Dr. Ulrich Specks to discuss the use of rituximab in other autoimmune diseases with renal involvement.

Dr. Ulrich Specks: In the interest of time, I will focus my presentation on the use of rituximab for anti-neutrophil cytoplasmic antibodies (ANCA)-associated vasculitis (AAV). AAV comprises three heterogeneous syndromes: Wegener’s granulomatosis (WG), microscopic polyangiitis (MPA), and the Churg-Strauss syndrome (CSS) (84). These three multisystem disorders are characterized by necrotizing small-vessel vasculitis with predilection for the kidneys, lungs, and peripheral nervous system that share the occurrence of ANCA in most patients at the time of initial presentation (85–87). The use of CYC has substantially improved the prognosis of these patients, turning a previously universally fatal illness into a chronically relapsing disease process (88); however, approximately 10% of patients fail to respond to CYC therapy. Furthermore, the toxicity of CYC therapy, including malignancies, infertility, and infection, may limit or prohibit its use in some patients (89). Consequently, better treatment alternatives are needed.

B lymphocytes play a critical role in the regulation of immune responses, are responsible for the production of antibodies, and are involved in the pathogenesis of autoimmune diseases, including, as discussed by Dr. Fervenza, MN (90). B lymphocytes are also responsible for the production of autoantibodies including ANCA, which have been implicated in the pathogenesis of several forms of vasculitis (91). The rationale for the first use of rituximab in a patient with chronically relapsing severe refractory WG included the following observations and hypotheses. First, higher frequencies of activated peripheral blood B cells are associated with disease activity and severity. Second, the most effective agent for WG (CYC) has profound effects on B cells. Third, the hypothesis that ANCA play a significant role in disease pathogenesis and evidence that autoantibodies are produced by short-lived plasma cells, which are the progeny of antigen-specific B cell precursors (92). Our index case was a 66-yr-old man who had received a diagnosis of WG in 1994. During treatment of disease flares, he had developed CYC toxicity, precluding its subsequent use, and prednisone in combination with azathioprine or MMF failed to restore remission. Treatment with four weekly doses of rituximab at 375 mg/m² was initiated on a compassionate-use basis under the hypothesis that lymphocyte depletion would result in rapid removal of potentially pathogenic ANCA. By the time of the fourth infusion, ANCA were undetectable in the serum, and 2 mo later, the patient was in clinical remission (92). Nine months after completion of the first course of rituximab, the patient’s ANCA began to rise, and he was retreated with another course of rituximab (this time without prednisone). To date, this patient has received six courses of rituximab and remains in remission.

This initial success prompted us to use the same rituximab regimen (four weekly courses of 375 mg/m²) in an additional 10 patients with PR3-ANCA–positive WG who were resistant or had contraindication to the use of CYC (93). The majority of these patients had received intravenous MTP, and three had also received plasma exchange preceding rituximab therapy to control life- or organ-threatening disease manifestations. Clinical remission accompanied by a significant decrease in ANCA levels was induced in all patients, and glucocorticoids were discontinued in all over the course of 5 mo. Two patients experienced minor flares, at 7 and 12 mo, which responded to re-treatment with rituximab and prednisone. Three patients were re-treated prophylactically without glucocorticoids for rising ANCA titers and remained in remission. Adverse effects were minimal and mostly related to the first infusion.

Encouraged by this favorable experience, we conducted a prospective, open-label, pilot trial of rituximab in conjunction with a strictly protocolized oral glucocorticoid tapering regimen (94). Ten patients with active severe refractory ANCA-positive WG or MPA were treated with the lymphoma dosing regimen of rituximab. Again, all patients achieved remission by 3 mo. One patient had a relapse after peripheral blood B lymphocytes and ANCA returned and was re-treated successfully with prednisone and rituximab. Five patients were re-treated with rituximab alone in the face of rising ANCA levels and remained in remission. Adverse effects again were minimal.

A number of other case reports and small case series of patients with WG, MPA, and CSS who received treatment with rituximab have been published recently (95–100). All of these reports support the use of rituximab for the treatment of refractory AAV.

To date, only a couple of reports have cast doubts on the efficacy of rituximab in refractory WG (101,102). Aries et al. (101) reported on patients of whom only two achieved clinical remission. The nonresponders had severe granulomatous orbital disease complications (101). Similarly, Omdal et al. (102) reported on two patients with granulomatous retro-orbital and sinus masses who seemed unresponsive to rituximab. This is different from our experience, which indicates that both vasculitic and granulomatous disease manifestations respond to rituximab (93,94). Hence, factors other than differences in disease manifestations may explain the observed differences in outcomes (94).

It should be emphasized that the exact mechanisms by which rituximab exerts efficacy in AAV remain poorly understood. Our original “ANCA-centric” hypothesis has recently been tempered by our treatment success with this agent in several ANCA-negative patients with WG (103). Finally, caution is advised about extrapolation from one autoimmune disease to another, because the pathogenic mechanisms, B lymphocyte depletion and autoantibody responses to rituximab therapy, are too heterogeneous among the different autoimmune diseases, particularly SLE, rheumatoid arthritis, and AAV (104,105).

The current reports suggest that rituximab is remarkably well tolerated by most patients. Adverse effects seem to be limited usually to mild infusion reactions occurring in up to 10% of patients and infections. Studies of rituximab use in lymphoma and a number of autoimmune diseases (e.g., rheumatoid arthritis, SLE) as well as our own experience in AAV
have revealed remarkably low infection rates even when rituximab has been used in combination with CYC or methotrexate (93,94,106,107). In patients with lymphoma and in patients with AAV, treatment with rituximab had minor effects on total serum Ig levels, and acquired humoral immunity is preserved after rituximab therapy (93,94,108). Our long-term experience over a mean of 35 mo of follow-up in 28 patients who received at least two repeated courses of rituximab for their refractory, chronically relapsing WG suggest that long-term B lymphocyte depletion is also a promising novel approach to remission maintenance associated with few adverse events (109). There is, however, some recent concern with the role of rituximab in the development of unusual infections, such as progressive multifocal leukoencephalopathy (PML) following the reports of two cases of PML resulting in death in patients who received rituximab for treatment of SLE (110). PML is a rare, progressive, demyelinating disease of the central nervous system that usually leads to death or severe disability. The disease is caused by activation of the JC virus, a polyomavirus that resides in latent form in up to 80% of healthy adults. PML has been reported in the literature in HIV-positive patients, immunosuppressed cancer patients, organ transplant recipients, and patients with autoimmune disease, including SLE and AAV, who had never received rituximab, suggesting that the overall immunosuppression rather than a particular agent is responsible for the development of PML (111,112).

I would like to conclude by saying that a pathogenic role for B lymphocytes in the pathogenesis of autoimmune diseases, including AAV, is emerging. Preliminary data indicate that B lymphocyte depletion with rituximab seems to be an effective and safe mechanism-based treatment approach for remission induction and remission maintenance in refractory AAV. Even repeated courses of rituximab therapy seem to be associated with surprisingly few infections. Whether B cell depletion can replace CYC as first-line therapy for patients with newly diagnosed AAV remains to be seen. We are currently conducting a randomized, double-blind, double placebo-controlled, multicenter trial in 200 patients with AAV. This trial is designed to evaluate the efficacy and safety of rituximab for remission induction in severe AAV in comparison with CYC (http://www.clinicaltrials.gov). Given the substantial morbidity and adverse effects associated with the use of CYC, rituximab may represent a promising alternative for the treatment of AAV.

Concluding Remarks

Idiopathic MN is a glomerular disease usually of abrupt onset and associated with the NS. Control of the NS, specifically CR or PR, is associated with prolonged renal survival and a slower rate of renal disease progression. There are no standard or universal first-line specific therapeutic options for idiopathic MN. Supportive or conservative care should be given in all cases and should include the use of ACEi and ARB therapy as well as a lipid-lowering agent. In patients who are at low risk for progression, this approach should suffice, given their excellent prognosis. These patients need to be followed long term to ensure that there is no disease progression. On the other hand, patients at medium or high risk for progression are candidates for immunosuppressive therapy, for there is overwhelming clinical evidence that higher sustained levels of proteinuria predict more rapid decline in renal function, more pronounced tubulointerstitial injury, and eventual kidney failure. It is also worth emphasizing that patients with persistent NS proteinuria have significant abnormalities in their lipid profile, and although these abnormalities can be improved by the use of an hepatic hydroxymethyl glutaryl-CoA reductase inhibitor, it will not be completely corrected unless NS goes into remission.

Thus, allowing a heavy proteinuric state to remain long term is likely to place the patient at an increased risk for cardiovascular complications. As illustrated by the case I presented earlier, nephrotic patients are also at risk for thromboembolic events, with an incidence as high as 50% in patients with severe MN being reported. These events are associated with a mortality rate as high as 42% in high-risk patients. These data emphasize that these life-defining events, in addition to the potential renal failure, are common in these patients. Therefore, even if the main benefit of immunosuppressive therapy is to speed up the induction of a remission that may have occurred spontaneously, it may still have value in the long term. A treatment algorithm that combines the predictive factors and best evidence for immunosuppressive therapy is presented in Table 1. The recommendation is based on evidence from trials conducted in patients in these respective categories, but the physicians must take into account the individual patient and his or her wishes to make the best decision regarding which therapy should be initiated. These treatments are not mutually exclusive and may follow one after the other (with a drug holiday) if the first one chosen does not succeed in reducing the proteinuria to the desired range and/or adverse effects make completion of a course of therapy untenable. Patients who do not respond well or relapse after a first course of immunosuppressive therapy may benefit from a second course of immunosuppression. It should be noted that I have made no reference in Table 1 to the use of corticosteroids because, in my opinion, the evidence to date does not support the widespread use of oral corticosteroids as a single agent for the treatment of MN; however, some of my distinguished colleagues may still recommend a “trial” of corticosteroids in patients with newly diagnosed MN and NS. Preliminary evidence on the use of anti-CD20 antibodies suggests that this is another agent that may be as effective and safer for the treatment of MN and AAV than our current regimens; however, rituximab is very expensive and not covered by any insurance policy, and before it can be recommended for routine use, it needs to be evaluated by rigorously conducted, prospective, controlled, randomized trials that will also look for factors that may determine its efficacy in patients with MN. Additional information will also be needed regarding long-term safety and efficacy (e.g., absence of relapses and serious infections, including PML) before it can be recommended as first-line therapy for patients with MN. Patients with severe renal insufficiency (serum creatinine ≥3 mg/dL) are less likely to benefit from immunosuppression therapy, and the risk of treatment is significantly higher, and these patients should be considered for conservative therapy only and plans made for transplantation in the future.
Table 1. Membranous nephropathy: Treatment recommendations

<table>
<thead>
<tr>
<th>Risk Level</th>
<th>Proteinuria/Clinical Function</th>
<th>Therapy Options</th>
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<tbody>
<tr>
<td>Low risk (&lt;4 g/24 h and normal renal function)</td>
<td>Conservative therapy</td>
<td>ACEi with or without ARB, lipid control with HMG-CoA reductase inhibitor, dietary protein restriction (0.6 to 0.8 g/kg ideal body wt per d), dietary NaCl intake (goal is 2 to 3 g of Na) to optimize antiproteinuric effects of ACEi and ARB BP &lt;125/75 mmHg, smoking cessation, reduce obesity, continue to monitor</td>
</tr>
<tr>
<td>Medium risk (&gt;4 to &lt;8 g/24 h and normal renal function)</td>
<td>Conservative therapy, initially; patients with proteinuria &gt;4 g/24 h after 6-mo observation period should be considered for additional immunosuppressive therapy with cyclosporine, tacrolimus, MMF, cytotoxic/corticosteroid combination, rituximab</td>
<td></td>
</tr>
<tr>
<td>High risk (≥8 g/24 h with or without renal insufficiency)</td>
<td>Conservative therapy, for &lt;6 mo; patients with proteinuria ≥8 g/24 h or decreasing renal function should be considered for additional immunosuppressive therapy with cyclosporine, tacrolimus, MMF, cytotoxic/corticosteroid combination, rituximab</td>
<td></td>
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*Adapted from reference (19). ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; HMG, hydroxymethyl glutaryl; MMF, mycophenolate mofetil.

Avoid dihydropyridine calcium channel blockers because they are not antiproteinuric. If needed for BP control, use a nondihydropyridine calcium channel blocker.

Cigarette smoking increases proteinuria and is associated with faster disease progression.

Obesity may cause glomerulomegaly and proteinuria. Weight loss reduces proteinuria.

Questions and Answer

Dr. Ajay K Singh (Brigham and Women’s Hospital and Harvard Medical School, Boston, MA): Thank you, Fernando, for an exceptional discussion. I would also like to thank our host, Prof. Rainer Oberbauer from Krankenhaus der Elisabethinen here in Linz Austria, for hosting this CJASN Clinical Conference. I have a comment and then a question. I noticed that in the case series you presented, several patients had progressive reduction in proteinuria that lasted several months after the last dose of rituximab. Given this observation, is rituximab retreatment in membranous necessary?

Dr. Fernando Fervenza: This is an excellent question for which I do not have a clear answer. Per protocol, patients who at 6 mo had recovered their circulating CD20+ B cell count but had proteinuria >3.5 g/24 h got a second identical course of rituximab. Would a patient who had a baseline proteinuria of 9 g/24 h and at 6 mo it had dropped to 4 g/24 h have continued to lower proteinuria over time without the second course of rituximab? I would guess the answer to be yes, but this is a question that was not addressed by the study.

Prof. Gert Mayer (Medical University Innsbruck, Austria): Is sequential administration of rituximab in primary nonresponders indicated?

Dr. Fernando Fervenza: This is another important question. At 6 mo, seven patients had not responded to treatment, meaning that their proteinuria was basically identical to their baseline levels. They all got re-treated, and two of them responded with proteinuria <1 g/24 g 12 mo after the second infusion. Thus, failure to respond to one course of rituximab makes it unlikely that a response will occur after a second treatment, but, as suggested by these two patients, we cannot state that for sure. It would be great if we could have markers that could predict which patient is likely to respond to rituximab, and we are trying to address this issue in a new study we are currently conducting (http://www.clinicaltrials.gov/ct/show/NCT00405340?order = 3).

Dr. Sabine Horn (Medical University Graz, Austria): How many patients developed antibodies against rituximab in your case series?

Dr. Fernando Fervenza: Thank you very much for raising this point. The formation of human antichimeric antibodies (HACA) may be induced by administering any chimeric mAb such as occur with the anti-TNF mAb infliximab; however, the formation of HACA after rituximab therapy is relatively uncommon. In a phase II trial of 58 patients with non-Hodgkin’s lymphoma who received rituximab (375 mg/m²), no patient developed HACA (113). The rare occurrence of HACA after rituximab therapy may be related to the main effect of the drug: The abolition of primary and memory humoral responses (114). On the other hand, the underlying disease itself may affect the propensity for antibody formation as reported by Looney et al. (115), who found detectable HACA in 30% of patients with SLE treated with rituximab. Similarly, Smith et al. (107) recently reported finding HACA in five of 14 patients with AAV. It is possible that other factors, such as the ethnic make up of the patient population and/or the use of concomitant cytotoxic or immunosuppressive agents, may also affect development of HACA. In our study, immunodepletable HACA was detected in six patients at various time points. In one patient with the lowest antibody level, HACA became negative with follow-up. In the other five patients, HACA remained positive to the end of the study.

Dr. James Pattison (Consultant Nephrologist, Guys Hospital, London, UK): Could you discuss the issue of HACA in rituximab-treated membranous patients?

Dr. Fernando Fervenza: We tested for the development of these antibodies because their appearance may affect the efficacy of rituximab. Similarly, although such antibodies are not...
associated with clinical symptoms (106,115), after repeated drug administration, they may contribute to adverse effects or retard B cell depletion. In the Smith et al. study mentioned earlier, relapses of AAV were more common in HACA-positive patients (five of five) than in HACA-negative patients (four of nine), and in two patients with the highest HACA titers, one patient develop a severe infusion reaction and one failed to achieve complete B cell depletion (107). In our study, we found no correlation among the presence of HACA, the degree of B cell depletion, and the development of adverse effects or between our responders and nonresponders; however, these observations are inconclusive, and future studies are needed to define further the clinical significance of HACA. Ocrelizumab, the new humanized anti-CD20 mAb, may ameliorate this potential problem.

Dr. Luana Pillon (Assistant Professor, New York University School of Medicine, Director Dialysis, VA New York Harbor Health Care System Hospital): Would there be any utility in determining the profile of the IgG subclasses in these patients? Dr. Fernando Fervenza: As you are aware, immunofluorescence examination of renal biopsies by Noël et al. (116) and Doi et al. (117) found IgG4 and IgG1 to be the predominant Ig subclass in the glomerular deposits in patients with idiopathic MN, with neither IgG2 nor IgG3 deposits detectable in any of these patients, whereas all four IgG subclasses (predominant IgG1 and IgG3) were detected in patients with membranoproliferative glomerulonephritis and lupus nephritis. On the other hand, Bannister et al. (118) found IgG3 and IgG4 to be the predominant Ig subclasses in patients with MN. Does the type or the amount of the deposited IgG subclass affects a patient’s response to rituximab? I do not know the answer, but we will be addressing this question in our ongoing study.

Acknowledgments

We thank Dr. Daniel Cattran for helpful comments to the manuscript. The studies with the use of rituximab were supported by unrestricted research grants from Genentech Inc. (South San Francisco, CA) and Biogen Idec (San Diego, CA) to F.C.F. and U.S. and by UL1-RR24150. The studies with the use of rituximab were supported by unrestricted research grants from Genentech Inc. (South San Francisco, CA) and Biogen Idec (San Diego, CA) to F.C.F. and U.S. and by UL1-RR24150. We thank Dr. Daniel Cattran for helpful comments to the manuscript. The studies with the use of rituximab were supported by unrestricted research grants from Genentech Inc. (South San Francisco, CA) and Biogen Idec (San Diego, CA) to F.C.F. and U.S. and by UL1-RR24150.

Disclosures

F.C. and U.S. have served as members of board advisory panels and have received investigator-initiated research grants from Genentech (South San Francisco, CA) and Biogen Idec (San Diego, CA).

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