

Novel therapies for membranous nephropathy: focus on rituximab

Paolo Cravedi

Department of Medicine and Transplantation, Azienda Ospedaliera Ospedali Riuniti Bergamo, Bergamo, Italy

Submitted: 15 January 2009

Accepted: 25 January 2009

Arch Med Sci 2009; 5, 3A: S 459–S 465
Copyright © 2009 Termedia & Banach

Corresponding author:

Paolo Cravedi, MD, PhD
Mario Negri Institute
for Pharmacological Research
Via Gavazzeni 11
24125 Bergamo, Italy
Phone: +39 035 433 34 05
Fax: +39 035 433 33 70
E-mail: p.cravedi@gmail.com

Abstract

Idiopathic membranous nephropathy (IMN) is the most frequent cause of nephrotic syndrome in adults and may progress to end-stage kidney disease in 30 to 40% of patients over 5 to 15 years. Until recently, the only therapeutic options were based on the use of non-specific immunosuppressants that, however, do not appreciably ameliorate patient and kidney outcome compared to placebo or no treatment. They also have a significant toxicity that may offset the benefits of proteinuria reduction. Thus, more specific and less toxic treatments are needed. Availability of rituximab, a chimeric monoclonal antibody targeting the CD20 antigen on B cells, offered a novel, selective and safer treatment for the disease. Rituximab depletes precursors of aberrant plasma cells, with secondary inhibition of the neoformation of autoantibodies possibly involved in the pathogenesis of the disease. Other treatments have been recently introduced in clinical practice that will hopefully help improve outcomes of the subset of patients with progressive disease.

Key words: membranous nephropathy, nephrotic syndrome, rituximab, mycophenolate mofetil, ACTH.

Introduction

Idiopathic membranous nephropathy (IMN) represents the leading cause of nephrotic syndrome in adults [1]. Although its pathogenesis is still only partially defined, the disease process appears to be initiated by the production of autoantibodies by aberrant plasma cell clones and the deposition of immune complexes in the outer space of the glomerular basement membrane [2]. As a consequence, the glomerular basement membrane thickens and loses its sieving function, resulting in increased permeability to circulating macromolecules and unrestricted glomerular ultrafiltration of plasma proteins, growth factors, complement components and inflammation mediators (Figure 1). These, in turn, sustain a chronic inflammatory process that accelerates tissue damage, with progressive scarring and the irreversible loss of kidney function in the most severe cases [3].

Despite an apparently similar pathology, the disease may have quite different outcomes from patient to patient. It may be benign or indolent in most of the cases, with a rate of spontaneous complete or partial remission as high as 60% [4], but in 30 to 40% of patients, it may also result in progression to end-stage renal disease (ESRD) in 5-15 years [5]. Discovering the predictors of progression is, therefore, instrumental to shape interventions to individual patients who are at increased risk and may benefit the most from effective treatment. Indeed, serious toxicity of steroids and immunosuppressive

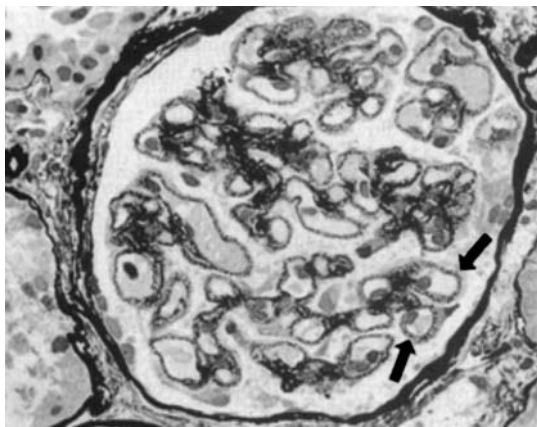


Figure 1. Glomerular pathology in idiopathic membranous nephropathy. A representative glomerulus showing diffuse spikes meaning from the outer surface of the basement membrane

drugs might be justified in those predicted to progress to ESRD, but would unnecessarily offset the potential benefit of proteinuria reduction in those with stable renal function.

Since the seminal studies by Heymann *et al.* depicted the autoimmune nature of the disease, the role of different immunosuppressive regimens, including glucocorticoids, alkylating agents (chlorambucil and cyclophosphamide), azathioprine and cyclosporine [6], has become one of the most exciting and controversial subjects of debate among nephrologists.

The inadequacy of available treatments has fuelled the research for innovative and safer immunosuppressive regimens, possibly targeted to specific disease mechanisms, in order to maximize the benefits and minimize risks. In this line of research, mycophenolate mofetil (MMF) [7], adrenocorticotrophic hormone (ACTH) [8], and, in particular, the monoclonal chimeric antibody rituximab targeted toward plasma cell precursors – CD20 B cells – have been used with the aim of inhibiting the synthesis of pathogenetic antibodies [9]. These promising options might help to improve outcomes of thousands of IMN patients worldwide.

Natural history

Data on the natural history and progression toward ESRD of IMN are to some extent discordant between studies, possibly because of the heterogeneity of populations and treatments in different series [1, 4, 5]. This is an issue of major clinical relevance, as clear disease prognosis characterization is crucial to establish whether it can be appreciably affected by therapy. In a series of 100 consecutive patients who had never received steroids or immunosuppressive agents, Schieppati *et al.* reported that 65% of them had a spontaneous remission and 88% still functioning kidneys at

5 years follow-up [4]. According to this study, only a minority of patients with progressive disease would represent the right target of specific therapy. Other reports with longer follow-up times found an 8- to 10-year incidence of ESRD in the range 20-30% [10-12]. However, all reports consistently found remarkably good outcomes, with survival rates of around 100% [12, 13] in those who never developed nephrotic syndrome. In such cases, immunosuppressive therapy should be avoided, as the potential adverse events would overwhelm the limited margins of improvement expected for a disease with a spontaneous good prognosis.

Thus, intervention should be limited to close monitoring and counselling, and the conservative treatment of risk factors such as hypertension, proteinuria and dyslipidaemia until the disease spontaneously fades away. On the other hand, older and male patients with long-lasting proteinuria in the nephrotic range [14], impaired glomerular filtration rate, or interstitial fibrosis at the time of diagnosis [15], increased urinary excretion of IgG [16], α 1 microglobulin [16], β 2 microglobulin [17], or complement components [18], are at high risk of progression and have a lower likelihood of spontaneous remission [19]. The challenge for the nephrologist is to identify who, among these patients, may benefit from interventions targeting disease activity.

Non-specific immunosuppressive strategies for patients with idiopathic membranous nephropathy

Over time, a series of treatment protocols have been used based on different drugs with the common characteristic of exerting non-specific suppression of the immune system. Benefits and risks of these regimens are briefly reviewed.

Steroids

Steroids were the first immunosuppressive agents employed for the therapy of IMN. A study found that a 2- to 3-month course of prednisone markedly reduced the rate of progressive renal failure when compared with placebo [20]. However, such a benefit was not confirmed by two subsequent trials, although proteinuria decreased during the period of corticosteroid administration [10, 21].

Alkylating agents

In the following years, studies showed more consistent antiproteinuric effects when cytotoxic agents such as chlorambucil or cyclophosphamide were added to steroids. A randomized trial showed that two-thirds of patients with IMN receiving a regimen consisting of methylprednisolone alternated with chlorambucil every other month for

6 months achieved remission of nephrotic syndrome, compared with a quarter of controls who did not receive any therapy over a mean follow-up period of 5 years [22]. Data at 10 years showed even more consistent benefits: 63% of treated patients vs. 33% achieved disease remission, and only 8% of patients progressed to ESRD compared with 40% of controls [23]. Similar long-term outcomes have been recently reported in a randomized trial of 93 patients from India showing that, at 10 years, 72% of patients treated with alternated methylprednisolone and cyclophosphamide compared with 39% of controls on placebo achieved complete or partial remission, and 11% of patients compared with 35% of controls progressed to ESRD [24].

Despite the above evidence of efficacy, the risk for bone marrow depression and later complications such as opportunistic infections, lymphoproliferative disorders and cancer [6] downsized enthusiasm around these treatments.

A recent meta-analysis of 18 randomized studies including 1,025 patients evaluated disease outcome in patients receiving placebo or no therapy, compared with patients allocated to four different immunosuppressive regimens: i) steroids alone, ii) alkylating agents, such as cyclophosphamide and chlorambucil, either alone or in combination with steroids, iii) ciclosporin, either alone or in combination with steroids, and iv) azathioprine alone [6]. Overall, the above treatments appeared more effective than placebo or no treatment in achieving partial or complete remission of nephrotic syndrome. However, this finding was confounded by a statistically significant heterogeneity of the studies considered. When a random effects model was used to account for this, the superior effect of immunosuppressive therapy was not statistically significant. Consistently, the above treatments did not improve patient or kidney survival. When the effect of each individual therapeutic regimen was evaluated separately, oral glucocorticoids had no beneficial effect on any of the considered outcome variables. Alkylating agents were associated with more complete or partial remissions, but again the effect was not significant when a random model was applied to correct for the confounding effect of study heterogeneity. Moreover, these treatments were associated with a significant excess of serious adverse effects, such as leukopenia, infections and gastric discomfort. Within the class of alkylating agents, there was weak evidence of a relatively beneficial effect on partial or complete remission of nephrotic syndrome of cyclophosphamide treatment, compared with chlorambucil. Of interest, cyclophosphamide was associated with a statistically significantly lower rate of discontinuation due to adverse events than chlorambucil [6].

Calcineurin inhibitors

Some small studies have shown a beneficial effect of cyclosporine (CsA) over placebo in the induction of remission of nephrotic syndrome in IMN patients, but also a high rate of relapses after treatment withdrawal [25-27]. This implies that prolonged and probably lifelong therapy may be needed to maintain sustained remission, which raises concern not only for the risks of chronic immunosuppression, but also of the chronic nephrotoxicity of treatment that might offset the benefits expected from sustained proteinuria reduction [28]. Similar considerations appear to apply also to tacrolimus. Indeed, a recent, multicentre, randomized clinical trial showed a significantly higher incidence of partial or complete remissions in IMN patients treated with tacrolimus, compared with controls taking placebo, but, similarly to what was observed previously with CsA, almost 50% of patients relapsed after treatment withdrawal [26].

Rituximab

Background

Rituximab is a chimeric mouse/human monoclonal antibody directed against the CD20 antigen expressed on mature B cells. Following treatment with rituximab, B cells are prevented from proliferating and undergo apoptosis and lysis through complement-dependent and complement-independent mechanisms [28]. Notably, B cell depletion generally persists over 6-9 months in > 80% of patients. Thus, rituximab has been used to treat patients with IMN and nephrotic syndrome with the rationale of depleting activated B cell clones producing the autoantibodies involved in the pathogenesis of the disease [9]. However, rituximab might exert its therapeutic effect also by depleting CD20 cells infiltrating the kidney tissue, where they appear to sustain the immune response by acting as antigen-presenting cells [29].

Initial experience

The effect of four weekly infusions of rituximab (375 mg/m²) was evaluated in eight IMN patients with persistent nephrotic syndrome [9]. At week 20 post-treatment, albuminuria and albumin fractional clearance decreased by 70 and 65%, respectively, and serum albumin increased by 31% [9]. The effect was sustained over 1 year of follow-up, and was associated with a reduction in body weight, diastolic blood pressure and serum cholesterol [30]. Another group recently confirmed the antiproteinuric effect of rituximab in 15 IMN patients with proteinuria > 5 g/24 h, despite RAS inhibition therapy [31]. Single case reports have shown that rituximab may also be effective in patients who are unresponsive to steroids and chlorambucil [32, 33], or with

post-transplant recurrent membranous nephropathy [34, 35].

Predictors of response to rituximab therapy: the role of kidney lesions

Rituximab is effective in most IMN cases, but it does not reduce proteinuria to the same extent in all patients [31, 36]. The heterogeneous effect on urinary proteins is not explained by different effects on B lymphocytes that are promptly and persistently depleted from the circulation in all patients [36]. Thus, factors independent from the actual inhibition of B cell-dependent immunological pathways might underlie the different responses to rituximab in different individuals [28]. The severity of chronic lesions most likely plays a role. In fact, chronic glomerular and tubulo-interstitial (TI) changes at baseline biopsy significantly predicted the response to rituximab treatment [36]. Rituximab therapy halved proteinuria at 3 months in patients with limited histological changes [tubulo-interstitial (TI) score < 1.7], but had no appreciable effect in those with a score \geq 1.7. Outcome analyses of IMN patients prospectively allocated to rituximab treatment on the basis of a TI score of < 1.7 confirmed that less severe TI changes predicted a more consistent reduction in proteinuria.

Data in experimental animals may explain the above findings. When a kidney taken from a rat with active Heymann nephritis (the animal model of the disease) is transplanted into a normal syngeneic recipient to abrogate the immune pathway elicited by immunization with renal target antigen (s) [37], proteinuria from the donor kidney decreases but does not entirely disappear. Residual proteinuria is attributed to the chronic glomerular damage that is initially induced by the immunological insult and that may eventually progress independently of the immune process [37]. This interpretation is consistent with functional and morphometric data showing that the magnitude of urinary protein traffic in this model was strongly related to changes in the epithelial layer of the glomerular capillary wall, but not to subepithelial immune deposits [38].

Is remission of proteinuria after rituximab therapy paralleled by regression of histological changes?

In 7 patients with long-lasting IMN and nephrotic syndrome achieving stable complete remission of proteinuria [39], repeat biopsies showed an almost complete recovery from the structural changes, including extensive foot process effacement and loss of intact slit diaphragms in a high percentage of filtration pores observed at baseline evaluation, that is before rituximab

administration. This suggests that preventing the immunologically mediated injury allowed progressive restoration of the glomerular epithelial layer [39]. The correlation found between treatment-induced changes in albumin fractional clearance and number of intact slit diaphragms reinforced a causal link between restoration of glomerular sieving function and recovery of podocyte dysfunction. Interestingly, the strong glomerular IgG4 staining at baseline biopsies disappeared completely or almost completely in repeat biopsy, suggesting a specific pathogenic role of this IgG subclass (Figure 2, panel A). Indeed, this was not paralleled by a reduction of total IgG deposits, which might simply reflect non-specific deposition (Figure 2, panel B) [39].

Specific inhibition of the production of pathogenic autoantibodies, possibly of the IgG4 class, might explain the functional and structural effects of rituximab.

Safety

Rituximab is generally very well tolerated, but mild hypersensitivity reactions during infusion may happen in around 10% of patients [28]. Although long-term data on the theoretical risk of opportunistic infections and malignancies associated with rituximab are lacking, reports published so far on the use of this antibody in IMN are encouraging [28].

Of note, however, the Food and Drug Administration recently issued a warning on the potential relationship between rituximab therapy and progressive multifocal leukoencephalopathy induced by reactivation of the polyomavirus JC in patients with autoimmune diseases [30]. Although the independent role of rituximab in inducing this event is unknown, as the antibody was administered in addition to other immunosuppressive therapies, the possibility that previous exposure to other immunosuppressants may enhance the risk of serious adverse events cannot be excluded when rituximab therapy is taken into consideration.

The ideal dose of rituximab

Rituximab has been used by a growing number of centres to treat IMN and other autoimmune diseases, including autoimmune haemolytic anaemia, systemic vasculitis, systemic lupus erythematosus (SLE), and idiopathic thrombocytopenic purpura (ITP) [40].

So far, however, only limited attention has been focused on finding the ideal dose of rituximab to be used in autoimmune disorders. Beside the original four weekly 375 mg/m² infusion protocol proposed for lymphoma, other regimens have been employed, including schedules with up to 8 weekly 325 mg/m² doses. Serial CD20 counts, however, showed that in

patients with immune mediated diseases, unlike those with lymphoproliferative disorders, B lymphocytes can be fully depleted from the circulation after a single 375 mg/m² infusion [41, 42]. This led to the question whether further rituximab administrations may enhance the efficacy of treatment or, rather, may just increase the risk of adverse reactions or sensitization.

To address this issue, a prospective, matched-cohort study compared the safety/efficacy profile of a B cell-driven rituximab treatment with the standard four 375 mg/m² dose protocol in 36 IMN patients with long-lasting nephrotic range proteinuria refractory to conventional therapy [41]. Patients allocated to the B cell-driven protocol received a second infusion only if they had more than five B cells per mm³ of peripheral blood after the first rituximab administration, which occurred in only one of the 12 patients in this group [41]. Importantly, the B cell-driven approach was as effective as the standard four-dose protocol in inducing IMN remission, but was associated with fewer adverse events, required a lower number of hospitalizations, and was four-fold less expensive, allowing for more than €10,000 (approximately \$15,000) savings per patient [41]. Despite the small sample size and the relatively short follow-up period, this study provides a valuable strategy to titrate rituximab therapy and shows that lower than standard doses of this antibody might be enough for treating patients with IMN. However, randomized studies are needed to confirm these findings.

Other suggested treatments for idiopathic membranous nephropathy: mycophenolate mofetil and synthetic adrenocorticotrophic hormone

Mycophenolate mofetil

Mycophenolate mofetil (MMF), an ester prodrug of mycophenolic acid, suppresses the proliferation of T and B lymphocytes by inhibiting inosine monophosphate dehydrogenase, a crucial enzyme in the *de novo* pathway of purine synthesis in the S phase of the cell cycle [7, 43]. Compared with other antiproliferative agents such as cyclophosphamide, chlorambucil and azathioprine, MMF may cause less bone marrow depression and possibly fewer severe chronic complications [43]. Mycophenolate mofetil halved proteinuria in 6 out of 16 IMN patients with nephrotic syndrome refractory to corticosteroids, cytotoxic agents or CsA, and achieved partial remission in 2 additional patients [43]. Treatment was well tolerated and no patients had serious side effects over the observation period. A significant reduction of proteinuria while on MMF therapy was also observed in a retrospective analysis of

17 patients with IMN resistant to standard immunosuppressive therapy, but 3 patients had to discontinue treatment because of serious drug-related adverse events (erosive gastritis, pneumonia, and squamous cell carcinoma) [7]. Notably, a recent randomized study on 36 patients with IMN and nephrotic syndrome showed that MMF had no benefit over placebo on proteinuria reduction and was associated with an increased incidence of serious adverse events [44]. Thus, despite the rationale for

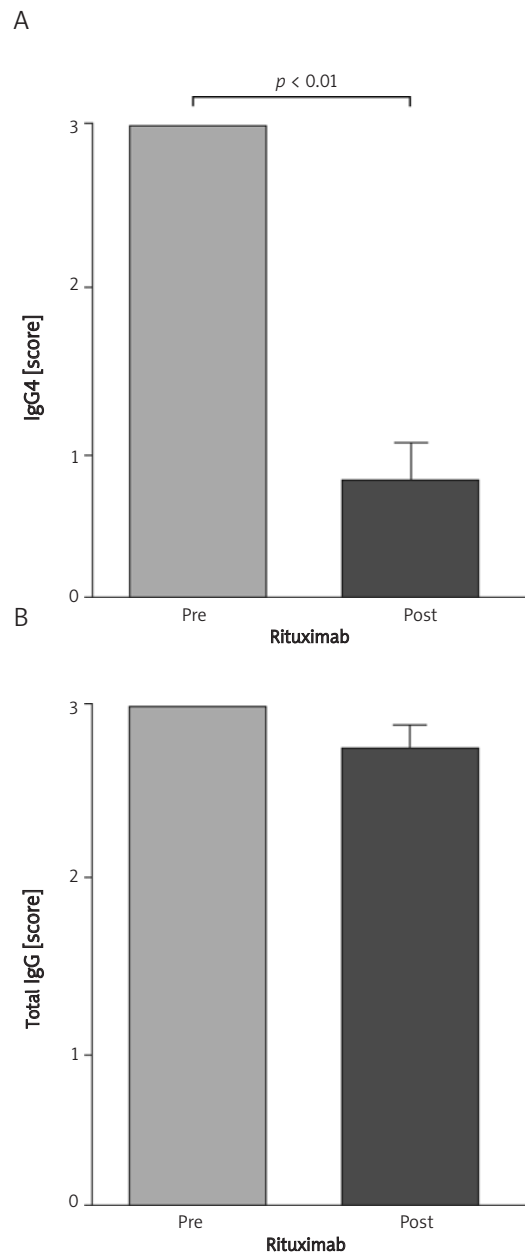


Figure 2. Glomerular IgG deposits in repeat biopsies before and after rituximab treatment. Glomerular staining for total IgG (panel A) and IgG class 4 (panel B) in 7 IMN patients with nephrotic syndrome before rituximab therapy and after a median period of 21 months of persistent proteinuria remission [39]

its use, the risk/benefit profile must be better established before MMF can be considered standard therapy for patients with IMN.

Synthetic adrenocorticotrophic hormone

One year of treatment with synthetic adrenocorticotrophic hormone (ACTH) decreased proteinuria and improved lipoprotein profiles in eight patients with IMN [8]. A randomized, pilot study of ACTH (twice a week for 1 year) compared to methylprednisolone alternated with a cytotoxic drug every other month for 6 months showed complete or partial remission in 14 out of the 16 patients on ACTH, compared with 15 out of the 16 patients on the steroid plus cytotoxic agent [45]. Adrenocorticotrophic hormone was also relatively well tolerated, with episodes of allergy requiring treatment withdrawal being reported in occasional cases. Thus, the risk-benefit profile of long-term ACTH therapy is worth investigating in appropriately designed, randomised, prospective trials. The effect of ACTH is likely independent from the induction of endogenous cortisol, as available data consistently show that corticosteroids alone, even at high doses, do not appreciably affect the outcome of the disease [20]. Adrenocorticotrophic hormone has a pronounced lipid-lowering effect mediated by modifications of apolipoprotein metabolism in healthy individuals [8] and restores glomerular expression of apolipoprotein J (clusterin), in patients with IMN [46]. Clusterin competes with the terminal components of complement C5b-9 for the same receptor in podocytes, namely megalin, which has been identified as the target antigen of the C5b-9-mediated injury in experimental models of membranous nephropathy [47]. Defective clusterin production might enhance C5b-9 binding to megalin, and this could sustain disease activity. On the other hand, increased clusterin production during ACTH therapy would decrease the amount of complement complex available for megalin binding, thus preventing glomerular damage [46].

Conclusions

Several immunosuppressive therapies have been suggested over the last decades to improve the outcome of patients with IMN, but benefits are uncertain. More specific treatments with drugs, such as rituximab, targeted to autoantibody production appear to have a more favourable risk/benefit profile compared to steroids or other immunosuppressants.

Single centre, underpowered studies will never allow optimal treatment of patients with IMN to be established. Large-scale, multicentre, international studies are urgently needed to optimize treatment at least of those at higher risk

of kidney failure or major complications of the nephritic syndrome.

References

1. Cameron JS. Membranous nephropathy and its treatment. *Nephrol Dial Transplant* 1992; 7 Suppl 1: 72-9.
2. Ronco P, Debiec H. Molecular pathomechanisms of membranous nephropathy: from Heymann nephritis to alloimmunization. *J Am Soc Nephrol* 2005; 16: 1205-13.
3. Nangaku M, Shankland SJ, Couser WG. Cellular response to injury in membranous nephropathy. *J Am Soc Nephrol* 2005; 16: 1195-204.
4. Schieppati A, Mosconi L, Perna A, et al. Prognosis of untreated patients with idiopathic membranous nephropathy. *N Engl J Med* 1993; 329: 85-9.
5. Honkanen E, Teppo AM, Meri S, Lehto T, Gronhagen-Riska C. Urinary excretion of cytokines and complement SC5b-9 in idiopathic membranous glomerulonephritis. *Nephrol Dial Transplant* 1994; 9: 1553-9.
6. Perna A, Schieppati A, Zamora J, Giuliano GA, Braun N, Remuzzi G. Immunosuppressive treatment for idiopathic membranous nephropathy: a systematic review. *Am J Kidney Dis* 2004; 44: 385-401.
7. Choi MJ, Eustace JA, Gimenez LF, et al. Mycophenolate mofetil treatment for primary glomerular diseases. *Kidney Int* 2002; 61: 1098-114.
8. 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: 1534-43.
9. Remuzzi G, Chiurciu C, Abbate M, Brusegan V, Bontempelli M, Ruggenti P. Rituximab for idiopathic membranous nephropathy. *Lancet* 2002; 360: 923-4.
10. 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: 210-5.
11. 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.
12. Donadio JV Jr, Torres VE, Velosa JA, et al. Idiopathic membranous nephropathy: the natural history of untreated patients. *Kidney Int* 1988; 33: 708-15.
13. 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: 127-34.
14. Pei Y, Cattran D, Greenwood C. Predicting chronic renal insufficiency in idiopathic membranous glomerulonephritis. *Kidney Int* 1992; 42: 960-6.
15. Reichert LJ, Koene RA, Wetzels JF. Prognostic factors in idiopathic membranous nephropathy. *Am J Kidney Dis* 1998; 31: 1-11.
16. 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: 240-8.
17. Reichert LJ, Koene RA, Wetzels JF. Urinary excretion of beta 2-microglobulin predicts renal outcome in patients with idiopathic membranous nephropathy. *J Am Soc Nephrol* 1995; 6: 1666-9.
18. Brenchley PE, Coupes B, Short CD, O'Donoghue DJ, Ballardie FW, Mallick NP. Urinary C3dg and C5b-9 indicate active immune disease in human membranous nephropathy. *Kidney Int* 1992; 41: 933-7.

19. du Buf-Vereijken PW, Branten AJ, Wetzels JF. Idiopathic membranous nephropathy: outline and rationale of a treatment strategy. *Am J Kidney Dis* 2005; 46: 1012-29.
20. 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: 1301-6.
21. 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: 133-56.
22. Ponticelli C, Zucchelli P, Imbasciati E, et al. Controlled trial of methylprednisolone and chlorambucil in idiopathic membranous nephropathy. *N Engl J Med* 1984; 310: 946-50.
23. 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: 1600-4.
24. Jha V, Ganguli A, Saha TK, et al. A randomized, controlled trial of steroids and cyclophosphamide in adults with nephrotic syndrome caused by idiopathic membranous nephropathy. *J Am Soc Nephrol* 2007; 18: 1899-904.
25. Cattran DC, Appel GB, Hebert LA, et al. Cyclosporine in patients with steroid-resistant membranous nephropathy: a randomized trial. *Kidney Int* 2001; 59: 1484-90.
26. Praga M, Barrio V, Juarez GF, Luno J. Tacrolimus monotherapy in membranous nephropathy: a randomized controlled trial. *Kidney Int* 2007; 71: 924-30.
27. Alexopoulos E, Papagianni A, Tsamelashvili M, Leontsini M, Memmos D. Induction and long-term treatment with cyclosporine in membranous nephropathy with the nephrotic syndrome. *Nephrol Dial Transplant* 2006; 21: 3127-32.
28. Ruggenenti P, Cravedi P, Remuzzi G. Latest treatment strategies for membranous nephropathy. *Expert Opin Pharmacother* 2007; 8: 3159-71.
29. Cohen CD, Calvaresi N, Armelloni S, et al. CD20-positive infiltrates in human membranous glomerulonephritis. *J Nephrol* 2005; 18: 328-33.
30. Ruggenenti P, Chiurciu C, Brusegan V, et al. Rituximab in idiopathic membranous nephropathy: a one-year prospective study. *J Am Soc Nephrol* 2003; 14: 1851-7.
31. Fervenza FC, Cosio FG, Erickson SB, et al. Rituximab treatment of idiopathic membranous nephropathy. *Kidney Int* 2008; 73: 117-25.
32. Cobo M, Hernandez D, Rodriguez C, Perez-Tamajon L. Successful therapeutic use of rituximab in refractory membranous glomerulonephritis. *Clin Nephrol* 2006; 66: 54-7.
33. Busch M, Gerth J, Ott U, et al. Treatment of idiopathic membranous nephropathy. Is the anti-CD20 antibody rituximab a reasonable option? *Med Klin (Munich)* 2008; 103: 519-24.
34. Gallon L, Chhabra D. Anti-CD20 monoclonal antibody (rituximab) for the treatment of recurrent idiopathic membranous nephropathy in a renal transplant patient. *Am J Transplant* 2006; 6: 3017-21.
35. Weclawiak H, Ribes D, Guilbeau-Frugier C, et al. Relapse of membranous glomerulopathy after kidney transplantation: sustained remittance induced by rituximab. *Clin Nephrol* 2008; 69: 373-6.
36. Ruggenenti P, Chiurciu C, Abbate M, et al. Rituximab for idiopathic membranous nephropathy: who can benefit? *Clin J Am Soc Nephrol* 2006; 1: 738-48.
37. Makker SP, Kanalas JJ. Course of transplanted Heymann nephritis kidney in normal host. Implications for mechanism of proteinuria in membranous glomerulonephropathy. *J Immunol* 1989; 142: 3406-10.
38. Shemesh O, Ross JC, Deen WM, Grant GW, Myers BD. Nature of the glomerular capillary injury in human membranous glomerulopathy. *J Clin Invest* 1986; 77: 868-77.
39. Ruggenenti P, Cravedi P, Sghirlanzoni MC, et al. Effects of rituximab on morphofunctional abnormalities of membranous glomerulopathy. *Clin J Am Soc Nephrol* 2008; 3: 1652-9.
40. Salama AD, Pusey CD. Drug insight: rituximab in renal disease and transplantation. *Nat Clin Pract Nephrol* 2006; 2: 221-30.
41. Cravedi P, Ruggenenti P, Sghirlanzoni MC, Remuzzi G. Titrating rituximab to circulating B cells to optimize lymphocytolytic therapy in idiopathic membranous nephropathy. *Clin J Am Soc Nephrol* 2007; 2: 932-7.
42. Looney RJ, Anolik JH, Campbell D, et al. B cell depletion as a novel treatment for systemic lupus erythematosus: a phase I/II dose-escalation trial of rituximab. *Arthritis Rheum* 2004; 50: 2580-9.
43. Shipkova M, Armstrong VW, Oellerich M, Wieland E. Mycophenolate mofetil in organ transplantation: focus on metabolism, safety and tolerability. *Expert Opin Drug Metab Toxicol* 2005; 1: 505-26.
44. Dussol B, Morange S, Burtey S, et al. Mycophenolate mofetil monotherapy in membranous nephropathy: A 1-year randomized controlled trial. *Am J Kidney Dis* 2008; 52: 699-705.
45. Ponticelli C, Passerini P, Salvadori M, et al. A randomized pilot trial comparing methylprednisolone plus a cytotoxic agent versus synthetic adrenocorticotrophic hormone in idiopathic membranous nephropathy. *Am J Kidney Dis* 2006; 47: 233-40.
46. Ghiggeri GM, Bruschi M, Candiano G, et al. Depletion of clusterin in renal diseases causing nephrotic syndrome. *Kidney Int* 2002; 62: 2184-94.
47. Orlando RA, Kerjaschki D, Farquhar MG. Megalin (gp330) possesses an antigenic epitope capable of inducing passive Heymann nephritis independent of the nephritogenic epitope in receptor-associated protein. *J Am Soc Nephrol* 1995; 6: 61-7.