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Review Article

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Membranous Nephropathy: Current Understanding in The Light of New Advances

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ABSTRACT

Membranous nephropathy is the most common cause of primary nephrotic syndrome in adults. The most important mechanism in its pathogenesis is loss of immune tolerance. New developments in membranous nephropathy are mostly related to the diagnosis and treatment of the disease, and until recently, the gold standard method in diagnosis was a kidney biopsy. In recent years, many membranous nephropathy-associated antigens and antibodies have been identified. The increased availability of these biomarkers is beneficial in predicting the treatment response, determining the treatment plan, and eliminating the necessity of kidney biopsy in the diagnosis of membranous nephropathy. Because of both the difference in treatment responses and the treatment-related side effects, membranous nephropathy treatment should be individualized. In addition, it is recommended to make a treatment plan by calculating the risk of progressive kidney failure of the disease. Parallel to the changes in diagnosis and follow-up, treatment plans in membranous nephropathy have undergone severe changes in recent years. As the autoimmunity targets in the pathogenesis of the disease become clearer, treatment has turned to more specific therapies that are more selective in targeting antibody-producing cells, such as rituximab. This article described the new developments in the pathogenesis, diagnosis, and treatment of membranous nephropathy.

Keywords: Anti-phospholipase A2 antibody, membranous nephropathy, nephrotic syndrome, rituximab

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INTRODUCTION

Membranous nephropathy (MN) is a non-inflammatory autoimmune disease defined by the presence of subepithelial immune deposits localized between the podocyte and the glomerular basement membrane (GBM) on electron microscopy examination. Although terms such as membranous glomerulonephritis or epimembranous glomerulonephritis were used to name the disease in the past, the term membranous nephropathy is often preferred today, especially because of its non-inflammatory character. It is the most common cause of primary nephrotic syndrome (NS) in adults, with an annual incidence of 1/100 000 cases. It is most often detected in the 40s and is more common in men than in women.[1,2] About 70%-80% of MN patients are classified as primary MN, while 20%-30% are classified as secondary MN.[3] The most common underlying causes of secondary MN are infections, drugs, malignancies, and autoimmune diseases. The frequency of secondary MN is higher in patients diagnosed with MN in childhood or advanced ages, and detailed research should be done on the underlying causes.

ETIOLOGY AND PATHOGENESIS

There are many mechanisms implicated in the pathogenesis of MN. In patients with an underlying genetic predisposition and/or immune dysregulation, predisposing factors such as infection, malignancy, or environmental

factors trigger a loss of tolerance for an autoantigen, resulting in B cell activation and autoantibody production. These autoantibodies damage the podocytes through complement-related and complement-independent mechanisms, resulting in the development of proteinuria.[4,5]

Early processes in elucidating the pathogenesis were presented with the Heymann nephritis model in the mouse. It has been shown that immune deposits accumulate in the subepithelial part of the GBM as a result of the binding of circulating immunocomplexes to antigens on the glomerular capillary membrane. Immune complex formations cause local activation of the complement system and result in complement-related cellular damage, GBM, and podocyte damage.[6] The injury process is chronic and ultimately results in severe proteinuria, which is the typical clinical manifestation of MN patients.

However, the podocyte protein targeted in the Heymann nephritis model is megalin, which has been identified in mice but is not expressed in humans. Megalin-like target antigens have been identified in humans in recent years. The first of these antigens was neutral endopeptidase (NEP). As a result of NEP-deficient mothers creating an antibody response in previous pregnancies, it has been shown that antibodies transferred from the mother cause an NEP-related MN process in infants.[7] The discovery of anti-NEP-related MN in neonates was the first demonstration that a pathogenic mechanism similar to the Heymann nephritis model was present in humans. However, these cases are rare and did not explain the majority of cases of MN seen in clinical practice.

This change in 2009 with the discovery of the M-type phospholipase A2-receptor 1 (PLA2R1), which accounts for 60%-70% of all cases of MN.[8] This seminal discovery was followed by a number of other targets such as the thrombospondin type-1 domain-containing 7A (THSD7A) protein, the neural epidermal growth factor-like 1 protein (NELL-1), and semaphorin-3B (SEMA3B), protocadherin 7A, and others that has revolutionized our understanding of the pathogenesis of MN.

MAIN POINTS

- Many membranous nephropathy (MN)-associated antigens and antibodies have been identified, and these antigens are beneficial in predicting the treatment response, determining the treatment plan, and eliminating the necessity of kidney biopsy in the diagnosis of MN.
- Membranous nephropathy treatment should be individualized, and it is necessary to make a treatment plan by calculating the disease risk of progressive kidney failure.
- As the autoimmunity targets in the pathogenesis of the disease become clearer, treatment has turned to more specific therapies that are more selective in targeting antibody-producing cells, such as rituximab.

The role of genetic predisposition in the development of MN has been clearly revealed. Two genetic locus encoding Major Histocompatibility Complex, Class II, DQ Alpha 1 (HLA-DQA1) and PLA2R, which cause genetic susceptibility to MN in the European race, were identified. The risk of developing MN increases 20 times in the presence of HLA-DQA1 and 4 times in the presence of homozygosity for PLA2R1 gene alleles.[9]

DIAGNOSIS

Membranous nephropathy patients often present with NS (proteinuria > 3.5 g/day and serum albumin <3.5 g/dL). Hypertension is present in approximately 30% of the patients at the time of diagnosis, and microscopic hematuria is common. Acute kidney injury is rare, and patients with acute kidney dysfunction are usually those with crescentic glomerulonephritis, acute interstitial nephritis, or kidney vein thrombosis.

Until recently, the gold standard diagnostic method in diagnosing the disease was a kidney biopsy. The term “membranous,” which gives the disease its name, refers to the diffuse thickening of the GBM, which is easily visible under the light microscope. However, glomeruli may look normal in early cases. Immunofluorescence microscopy shows granular deposits, most commonly with antihuman immunoglobulin G (IgG) and complement C3.[10] This produces a beaded appearance along the GBM (capillary wall), a pattern that is pathognomonic of MN on immunofluorescence. Staining kidney biopsies for IgG subclasses shows IgG4 to be more commonly expressed in primary MN and absent in MN secondary to malignancy. A full-house pattern of Ig staining (G, M, and A), including staining for C1q on immunofluorescence microscopy, suggests MN secondary to an autoimmune disease (e.g., Systemic Lupus Erythematosus [SLE]).

The most characteristic findings of the disease in electron microscopy are electron-dense accumulations on the outer surface of GBM with extensive foot process effacement.

While kidney biopsy is considered the gold standard diagnostic method in the diagnosis of most glomerular diseases, positive detection of antibodies against anti-PLA2R receptors in patients presenting with NS, who are not diabetic, has no evidence for a secondary disease (SLE, hepatitis, malignancy, drugs, and sarcoidosis), and patients who have a normal estimated glomerular filtration rate (eGFR) may not need a biopsy.[11]

However, kidney biopsy is mandatory in patients with NS who are anti-PLA2R negative or have signs of kidney disease other than membranous nephropathy (GFR less than 60 mL/min, diabetes, or evidence of secondary disease).[12] In the past, examining kidney biopsies by light microscopy, immunofluorescence, and electron microscopy was considered sufficient, but new approaches suggest staining antibodies at the tissue level and trying to detect antibody-associated MN. Immunohistochemical analysis with kidney biopsy for PLA2R should be performed on

patients who cannot detect circulating anti-PLA2R antibodies. Positive tissue-level staining may be diagnostic of antibody-associated MN in patients with false-negative results by serum enzyme-linked immunosorbent assay (ELISA) and immunofluorescence tests due to low titer antibody load. This may mean that in the early stage of MN, antibodies may not be present in the circulation, may only be found in tissue, and may become detectable after long-term follow-up.[13] There is not yet sufficient data to support that biomarkers other than anti-PLA2R (including THSD7A) are useful in diagnosing MN without kidney biopsy. All MN patients should be evaluated for secondary causes regardless of the presence of anti-PLA2R antibodies and/or anti-THSD7A antibodies.

In the diagnosis of MN, many antigens and antibodies specific to these antigens have been detected in recent years. Introducing these biomarkers is beneficial in predicting the treatment response and determining the treatment plan, as well as eliminating the necessity of kidney biopsy in diagnosing MN. These biomarkers include PLA2R1, THSD7A, NEP, NELL-1, SEMA3B, protocadherin 7 (PCDH7), neural cell adhesion molecule-1 (NCAM-1), and serine protease high-temperature recombinant protein A1 (HTRA1).[14-16]

Biomarkers Associated with Membranous Nephropathy

Following identification of the anti-PLA2R antibody with the target antigen PLA2R, a number of other targets have been identified.[8] These include THSD7A,[16] exostosin 1/exostosin 2 (EXT1/EXT2), NELL-1,[17] SEMA3B, NCAM-1,[18] HTRA1, and PCDH7.[19,20] Most recently, FAT atypical cadherin 1 (FAT1)-associated MN appears to be a unique type of MN associated with hematopoietic stem cell transplant.[20] However, 10%-20% of MN-related target antigens are still waiting to be discovered.[21] In approximately 70% of the patients with MN, the target antigen is PLA2R followed by NELL-1, PCDH7, THSD7A, HTRA1, SEMA3B (mainly in children and young adults), and NCAM-1. The target antigen remains unknown in the remaining 10%-15% of patients with MN. In ~20% of patients, MN occurs in association with other clinical conditions and is categorized as secondary. However, the concept of primary versus secondary MN has been challenged because in many instances clinical findings overlap between patients considered to have primary MN versus secondary MN. In addition, some patients with apparently secondary MN are also positive for PLA2R such as cases associated with hepatitis B or hepatitis C infection or sarcoidosis.[22,23]

Anti-Phospholipase A2 Receptor Antibody

Anti-phospholipase A2 receptor antibodies were first discovered in adult MN in 2009 and are positive in approximately 70% of MN patients.[8] In recent years, it has been recommended by the guidelines to measure anti-PLA2R antibodies in diagnosis, treatment decision, and follow-up.[13,24] High antibody levels predict poor treatment response, frequent relapse, progressive loss of kidney function, and disease relapse after kidney transplantation. At the same time, remission rates are lower in patients with positive antibodies, both spontaneously and under immunosuppressive therapy.[12,25,26]

The 2021 Kidney Disease Improving Global Outcomes (KDIGO) guidelines now state that kidney biopsy can be performed in the diagnosis of MN if there is an unexplained decrease in GFR, the expected response to immunosuppressive therapy is not achieved, or additional non-MN diagnoses are considered.[13]

Serum anti-PLA2R antibody levels are more closely correlated with disease activity and kidney function compared to PLA2R deposition in the glomeruli. Serum anti-PLA2R antibody levels may also be detected earlier in proteinuria, and decreased antibody levels may predispose to a decrease in proteinuria.[27] Therefore, it is mandatory to monitor serum antibody levels during treatment and follow-up.

Thrombospondin Type-1 Domain-Containing Protein 7A

Anti-thrombospondin type-1 domain-containing protein 7A antibodies are positive in approximately 10% of anti-PLA2R antibody negative cases and approximately 3% of all MN patients.[16] Both PLA2R and THSD7A are not only detected as circulating antibodies, but they can also be demonstrated at the tissue level in kidney biopsies. Antibody detection in the tissue is more sensitive, especially in cases with low serum levels and under treatment.[16]

Anti-THSD7A antibody has a sensitivity of 4% and a specificity of 99% in the diagnosis of primary MN and a sensitivity of 8% and a specificity of 100% in PLA2R-negative patients.[27] Although the findings suggest that anti-THSD7A antibodies have a high diagnostic value for PLA2R-negative primary MN and can be used as an adjunctive diagnostic method, there is currently insufficient data to support the application of anti-THSD7A antibodies as a diagnostic biomarker for MN instead of biopsy.[21] Anti-THSD7A antibody levels, like PLA2R, are closely related to treatment response and disease activity.[28]

Similar to the anti-PLA2R antibody, the anti-THSD7A antibody is mainly detected in primary MN, but it is not possible to use this antibody to differentiate between primary and secondary MN. The frequency of malignancy-related MN is higher in patients with THSD7A-associated MN; therefore, detailed malignancy screening should be performed.[29]

Exostosin 1/exostosin 2

Exostosin 1 and EXT2 were first demonstrated in PLA2R-negative patient biopsies in 2019. The most striking features of EXT1- and EXT2-positive patients are the presence of clinical and laboratory findings of autoimmune diseases (SLE) and IgM- and IgG1-predominant accumulation in biopsies. Exostosin 1 and EXT2 are the most common specific target antigens of PLA2R-negative MN and are especially detected in patients with secondary MN.[17]

Exostosin 1 and EXT2 are detected in approximately 30%-40% of secondary MN. Approximately 35% of EXT1- and

EXT2-associated secondary MNs are membranous-type lupus nephritis (LN). Therefore, EXT1 and EXT2 are considered major subtypes of secondary MN and can be used as potential markers of both secondary MN and LN.[17] Interestingly, patients with EXT1- and EXT2-positive LN show a less progressive disease course than negative patients and have a lower rate of progression to end-stage kidney disease.[30] To date, anti-EXT1 and EXT2 antibodies have not been detected in the peripheral circulation but have been identified only in kidney tissues. This indicates that the widespread use of EXT1 and EXT2 as non-invasive biomarkers in diagnosing MN is unlikely.[17]

Neural Cell Adhesion Molecule-1

Neural cell adhesion molecule-1 is another podocyte target antigen identified in membranous-type LN. It is found in the tissues of patients with membranous LN without positive staining in normal kidney tissues. Both NCAM-1 and EXT1/EXT2 are considered primary biomarkers of membranous LN.[18]

Neural Epidermal Growth Factor-Like Protein-1

Neural epidermal growth factor-like protein-1 is a positive biomarker, especially in malignancy-associated MN cases. The incidence of malignancy in patients with NELL-1-associated MN ranges from 11.7% to 33%. Membranous nephropathy may also occur before the detection of malignant tumors. Therefore, NELL-1-positive MN patients should be evaluated regularly to exclude the presence of malignancy.[18]

Protocadherin 7

The characteristics of PCDH7-associated MN are still not clearly defined. It is thought to be positive especially in patients with primary MN. The essential features of PCDH7-associated MN are the absence or deficient complement activation in these patients and the high frequency of spontaneous remission without needing immunosuppressive therapy.[19]

In addition, biomarkers such as SEMA3B, HTRA1 and netrin G1 (NTNG1) were also detected against podocytes[31,32] part from podocyte antigens, many new biomarkers have been discovered in the diagnosis of MN and are still being developed for clinical use. Among these, urinary lysosomal integrated membrane protein, alpha-1-antitrypsin, and aphamine are the most prominent.[33]

PROGNOSIS AND TREATMENT DECISION

Approximately one-third of patients with MN develop spontaneous remission within 1 year of diagnosis, and 20%-30% develop kidney failure within 10 years.[34] Because spontaneous remission is common in MN and there are side effects of immunosuppressive treatments, treatment must be individualized. It is important to assess the risk of kidney failure before deciding which patients are suitable for immunosuppressive therapy and the duration of treatment. On the other hand, the KDIGO 2021 guide recommends that patients be treated by classifying them according to the risk of kidney function loss.[13]

KDIGO 2021 glomerular disease management guideline suggested dividing patients into 4 classes as low, moderate, high, and very high risk before starting treatment in MN patients and making follow-up and treatment plans accordingly.[13]

- Low risk (patients with normal GFR, serum albumin >3 g/dL, if measured by bromocresol or >3.5 g/dL if measured by bromocresol green methods, and proteinuria <3.5 g/day or a decrease of more than 50% after 6 months of supportive treatment)
- Moderate risk (patients with normal GFR and proteinuria >3.5 g/day and less than 50% reduction after 6 months of supportive therapy)
- High risk (GFR <60 mL/min and/or patients with proteinuria >8 g/day despite 6 months of supportive treatment or GFR normal, proteinuria >3.5 g/day and proteinuria less than 50% after 6 months of supportive treatment and patients with 1 of the following findings: serum albumin < 2.5 g/dL, PLA2R > 50 U, urine α-1 microglobulin > 40 µg/min, β-2 microglobulin > 250 mg/d)
- Very high risk (life-threatening NS or rapidly progressive loss of kidney function)

Considering that up to 30% of the patients may go into spontaneous remission rates in MN (mainly patients who are anti-PLA2R negative, have sub-nephrotic proteinuria, or have low levels of anti-PLA2R antibodies levels (<50 RU/mL), it is reasonable to follow-up for 6 months with maximum tolerant anti-proteinuric therapy. However, patients with high proteinuria and high anti-PLA2R antibodies levels require reassessment before 6 months. Patients with documented 20% reduction in GFR in less than 24 months have an 84% probability of progression. Therefore, patients with NS who have impaired kidney function or do not respond significantly to conservative treatment should be promptly evaluated for immunosuppressive therapy.[13] In studies evaluating the relationship between the probability of spontaneous remission and anti-PLA2R levels, spontaneous remission is common with antibody levels below 40-50 U/mL; antibody levels above 150 RU/mL usually require an immunosuppressive therapy.[25,35] Patients with proteinuria >4 g/day after 6 months of conservative treatment had a 45% chance of spontaneous remission. It was found to be 34% in patients with proteinuria greater than 8 g and 20% in patients with anti-PLA2R antibody levels >275 RU/mL.[36,37] As such, the decision to initiate immunosuppresive therapy should be based on evaluating antibody levels, GFR changes during follow-up, degree of proteinuria, serum albumin levels, the presence of other factors (impact of the NS in patient's ability to conduct normal activities), as well as their changes in the follow-up.[13]

In patients who are anti-PLA2R positive, an anti-PLA2R-based therapeutic approach has the potential to significantly reduce treatment intensity and toxicity and improve the prognosis of MN. Because glomerular damage may take years to repair (if ever completely) following the disappearance of anti-PLA2R

antibodies, proteinuria (usually <3.5 g/day) may persist long term in patients who are in immunological remission. Therefore, in patients diagnosed with PLA2R-associated MN treatment, decision on immunosuppression cannot be based solely on proteinuria. When anti-PLA2R antibodies become undetectable in the blood, immunosuppressive therapy should be discontinued. In practical terms, this only applies to calcineurin inhibitors (CNIs), because both rituximab (RTX) and cyclophosphamide (CYC) have long-lasting effects that persist well after drugs are discontinued.[4] Proteinuria usually persists in patients who remain positive for anti-PLA2R antibodies after immunosuppressive therapy or are likely to relapse following reappearance of the antibodies.[38]

Anti-PLA2R antibodies should be measured at 3- to 6-month intervals during follow-up. Changes in anti-PLA2R antibody levels during follow-up contribute to the risk estimation. Loss of anti-PLA2R antibodies precedes clinical remission and should lead to avoidance of additional therapy.[13]

Remission Criteria

Although international guidelines have determined definitions of complete and partial remission, different remission criteria have been used in studies. These should be taken into account when evaluating the studies. While the The Membranous Nephropathy Trial Of Rituximab (MENTOR) study defined complete remission as proteinuria less than 300 mg/24 hours and serum albumin level more than 3.5 g/dL, it accepts partial remission as proteinuria between 0.3 and 3.5 g/24 hours, provided that there is at least 50% reduction from baseline.[39] On the other hand, Ramachandran et al[40] accepted patients with normal serum albumin (≥3.5 g/dL) and serum creatinine in complete remission if proteinuria was <500 mg/24 hours. They were considered to be in partial remission if the proteinuria decreased by less than 50% compared to the baseline value or was between 0.5 and 2 g. Similarly, the criteria for a complete remission for the Sequential Treatment with Tacrolimus and Rituximab Versus Alternating Corticosteroids and Cyclophosphamide in PMN (STARMEN)[41] study are proteinuria below 0.3 g/24 hours and stable kidney function with GFR ≥ 45 mL/min/1.73 m^2. For partial remission, it is a stable kidney function (GFR ≥ 45 mL/min/1.73 m^2) when proteinuria is less than 3.5 g/24 hours, with a greater than 50% reduction from baseline in proteinuria. For the Rituximab versus Steroids and Cyclophosphamide in the Treatment of Idiopathic Membranous Nephropathy (RI-CYCLO)[42] study, complete remission was defined as proteinuria ≤0.3 g/24 hours and partial remission as ≤3.5 g/24 hours with at least 50% reduction.

TREATMENT

One of the main elements of treatment in all MN patients is supportive treatment. Low protein diet (0.8-1 g/kg/day), low sodium intake (<2 g/day), keeping systolic blood pressure below 120 mmHg, diuretic therapy in patients with edema, medical treatment for hyperlipidemia, and anticoagulant therapy in appropriate patients are the main supportive treatments of glomerular diseases. More importantly, all MN patients should receive renin–angiotensin system inhibitor therapy at the maximum dose they can tolerate, unless there is a contraindicated situation.[13]

Until recently, immunosuppressive treatment in MN consisted of corticosteroids combined with a cytotoxic agent (CYC or chlorambucil) of a CIN.[43] The combination of CYC with glucocorticoid (GC) has replaced chlorambucil in combination therapies because it has similar efficacy and lower side-effect profile to chlorambucil.[44] In the following years, CYC became the treatment regimen recommended by the guidelines, both by demonstrating its effectiveness in patients with impaired kidney function and by demonstrating that high remission rates were achieved with oral therapy.

Other treatment agents used in the treatment of MN for many years are CNIs. The efficacy of cyclosporine in the treatment of MN was initially demonstrated in a few studies with small numbers of patients, but very high recurrence rates were noticed when the drug was discontinued or when the dose was reduced.[45] Cyclosporine + GC combination achieved higher remission rates compared to GC treatment alone.[45] High remission rates have also been reported with the use of tacrolimus, but, as with cyclosporine, the risk of relapse after discontinuation is very high (>35%).[46,47] Obtaining high remission rates with CNI also led to a comparison of these agents with CYC + GC combinations, which is the most popular MN treatment option. Studies have shown that tacrolimus combined with GC provides similar remission rates and similar times to reach remission but much lower long-term remission times than CYC combined with GC.[48]

In the following 10 years, many new treatment options were introduced, and the approach to MN treatment changed completely (Figure 1).

With a greater understanding of autoimmunity targets in MN, there has been a shift toward a more pathogenesis-based therapy targeting autoantibody-producing B cells using RTX rather than non-selective immunosuppressive agents such as alkylating agents and CNIs.[49] The effectiveness of RTX in MN was first demonstrated by Remuzzi et al[50] who treated 8 patients with MN with RTX 375 mg/m^2 weekly for 4 weeks and found that at 12 months, 2 patients had gone into complete remission, 3 patients in partial remission, and 3 patients had >50% reduction in proteinuria. These findings were confirmed in subsequent studies. Fervenza et al[51] reported complete or partial remission in 80% of patients treated with RTX (375 mg/m^2, weekly for 4 weeks, with retreatment at 6 months) after 2 years of follow-up. After the detection of anti-PLA2R antibodies, the relationship between immunological remission and RTX began to come to the fore. Beck et al[52] were the first to demonstrate that antibody response mirrored proteinuria response. Evaluating 25 patients with MN treated with RTX, these investigators

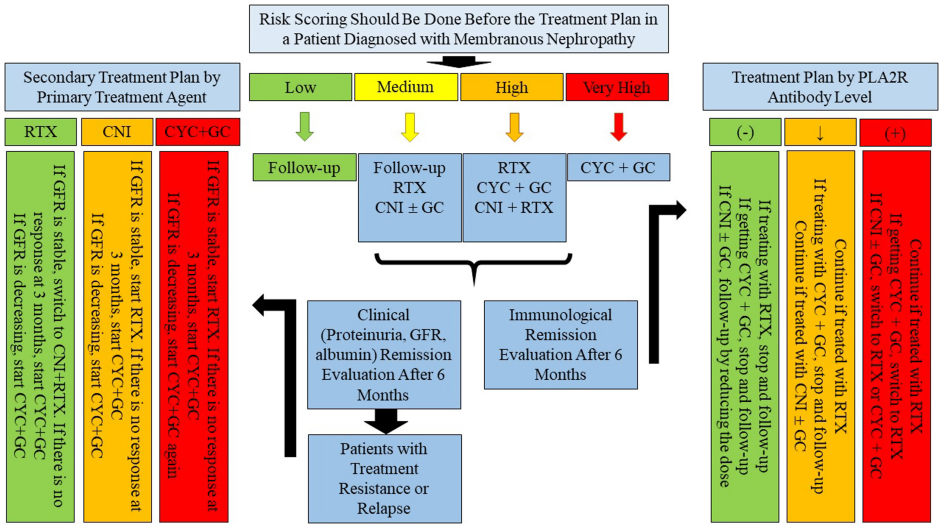

Figure 1. Membranous nephropathy follow-up and treatment chart. CNI, calcineurin inhibitor; CYC, cyclophosphamide; GC, glucocorticoid; GFR, glomerular filtration rate; PLA2R, anti-phospholipase A2 antibody; RTX, rituximab.

demonstrated that the disappearance of anti-PLA2R antibodies (immunological remission) was followed by remission in proteinuria, while the persistence of the antibodies in circulation was associated with persistence of proteinuria.

These pilot studies were followed by a number of recently conducted randomized controlled trials (RCTs). The Evaluate Rituximab Treatment for Idiopathic Membranous Nephropathy (GEMRITUX) study compared the efficacy of supportive therapy with 2 doses of RTX given adjunct to supportive treatment. While a post hoc analysis showed that the remission rate was higher in patients receiving RTX, there was no significant difference between the 2 groups regarding remission rates at the sixth month (the primary end point) and the development of side effects.[37] The MENTOR study compared the efficacy of RTX (1 g on day 1 and 15 followed by repeat treatment at 6 months in patients who had >25% reduction in proteinuria) with cyclosporine (3 g mg/kg/day; blood levels 125-175 ng/mL) in maintenance of remission. Remission rates were similar between both agents at 12 months, but maintenance of remission in the group receiving RTX was nearly 3 times higher than in those using cyclosporine at 24 months. Despite the high remission rates, the frequency of adverse events was lower in those receiving RTX.[39] The RI-CYCLO study compared RTX and CYC and revealed that the 2 agents had similar remission rates and safety profiles.[42] While all of the above studies were conducted in naïve patients, the STARMEN study focused on combination therapies in resistant NS. In the study, patients receiving GC + CYC and patients receiving tacrolimus + RTX treatment were compared. In the group of patients receiving GC + CYC, it was determined that the frequency of side effects increased, along with high remission rates and low recurrence rates.[41] This study is important because it demonstrates the effectiveness of the combination of GC and CYC in resistant patients as well as the effectiveness of the use of RTX in preventing relapses that occur during the discontinuation of tacrolimus therapy.

While RTX has proven efficacy in monotherapy, its effectiveness has also been tested with combination treatments, with remission rates of over 90% achieved with RTX + GC + CYC therapy.[53] As a result of a recent meta-analysis in which more than 500 patients were evaluated, RTX once again proved its efficacy in monotherapy. Despite the high remission rates in patients treated with RTX alone, no adverse effects were found on the frequency of side effects and the development of serious side effects.[54]

The efficacy of RTX in the treatment of MN and its low side-effect incidence has changed the treatment recommendations in both naive and treatment-resistant or relapsed patients. It is recommended to use the risk scale determined by the KDIGO 2021 guidelines to identify patients who are now candidates for immunosuppressive therapy.[13] According to this scoring, patients at low or moderate risk of disease progression are usually followed for 3-6 months with optimal supportive care. In low-risk patients, serum albumin levels are generally >30 g/L; if the patient's serum albumin is low, other causes of hypoalbuminemia must be excluded. There are no RCTs comparing the results of patients with non-nephrotic proteinuria who received and did not receive immunosuppressive therapy. However, studies showing favorable kidney outcomes in patients with non-nephrotic proteinuria who are not given immunosuppressive therapy indicate that immunosuppressive therapy is not essential for this patient group, given the potential risks associated with immunosuppressive therapy. Increasing proteinuria, rapid/progressive loss of GFR, or high/raising anti-PLA2R antibody levels at follow-up are defined as high-risk/progressive disease. If proteinuria is increased, GFR is decreased, or antibody

levels are increased, patients are assessed for treatment with RTX or CNI. Patients at high risk of disease progression should be treated with RTX ± CNI or CYC + GC. For patients at very high risk of disease progression (life-threatening NS or rapid loss of kidney function), the recommended treatment regimen is classical CYC + GC therapy,[13] although this is debatable, especially with the availability of new anti-CD20+ B cell monoclonal antibodies with more powerful B cell-depleting efficacy.[55]

Determining the indication for immunosuppressive therapy and risk stratification to guide treatment, as well as identifying individualized treatments based on risk score rather than standard treatment regimens for all patients, is a new concept compared to the 2012 KDIGO glomerulonephritis clinical practice guidelines.[13]

The use of mycophenolic acid analogs (MFAs) is one of the treatments that have been tried but without success other than standard treatment approaches. While the use of MFA does not show superiority in complete or partial remission rates compared to supportive treatment in studies, it is not recommended due to the increased risk of severe side effects.[56] Mycophenolic acid analog is more effective in combination with GC; however, immunological remission rates are much lower compared to standard treatment regimens, and relapse rates are significantly higher in patients treated with MFA.[57] Although higher remission rates were obtained in the combination of MFA with tacrolimus compared to the use of tacrolimus alone, any difference was not found between monotherapy and the combination in terms of recurrence.[58] In summary, although the effect of MFAs on proteinuria has been documented, at least in the short term and in the context of small studies, complete remission rates are low, relapses are frequent, and long-term benefits are not yet clear.

In the treatment of MN, the efficacy of plasmapheresis to eliminate pathogenic antibodies has also been a matter of interest following the success of antibody-associated therapies. This strategy has been successful in only a few patients, and the successful patients are those receiving various immunosuppressive therapies. Therefore, the precise role of plasmapheresis is still not fully defined.[59]

Frequent monitoring of anti-PLA2R antibody levels (every 2-3 months) helps evaluate response to immunosuppression and guide treatment in patients with MN.[39] Early initiation of immunosuppressive therapy is not recommended in patients who are anti-PLA2R antibody positive but have non-nephrotic level proteinuria, although some studies have shown that such patients often develop nephrotic level proteinuria.[25]

RELAPSE AND RESISTANT DISEASE

The definition of recurrent disease in MN has been made in many different ways by researchers, as in the criteria for remission. KDIGO guidelines recommend that if proteinuria is above 3.5 g/day before serum albumin returns to the normal range, this should be considered a resistant disease rather than a relapsed disease.[13] Relapse in patients with partial remission is with low serum albumin and increased proteinuria. In these cases, immunological follow-up is critical. If anti-PLA2R antibodies are still positive during complete remission, this indicates the need for attention for resistant disease.

In patients who are anti-PLA2R positive, treatment is aimed at immunological remission, i.e. negative anti-PLA2R antibodies by ELISA and Immunofluorescence (IF) testing. None of the patients with positive anti-PLA2R antibodies after completion of treatment remained in remission at 2 years.[38] Patients who show less than 50% reduction in antibody levels despite 6 months on adequate IS are considered resistant to therapy and should change immunosuppression regimen.[13]

Persistent proteinuria is not sufficient to define the resistant disease. If proteinuria persists despite normalization of serum albumin level and anti-PLA2R antibodies have disappeared, a secondary focal segmental glomerulosclerosis should be considered. Reemergence of or an increase in anti-PLA2R antibody titers precedes a clinical relapse by approximately 3 months.[60]

In refractory or relapsed patients, treatment depends on the severity of the GFR loss. If RTX is preferred as the second treatment, proteinuria and the response of anti-PLA2R antibodies should be evaluated every 2-3 months. If CYC was used in the first treatment in relapsed or resistant patients and if a repeat CYC is planned, the cumulative lifetime dose should be calculated. If fertility is to be preserved, the cumulative dose should not exceed 10 g. The cumulative dose should not exceed 36 g to limit the risk of malignancy. Experimental treatments (bortezomib, anti-CD38 therapy, and belimumab) can be tried in patients who do not respond to RTX and CYC treatments.[13] Newer anti-CD20 agents such as obinutuzumab have been shown to be effective in treatment of patients with MN resistant to RTX therapy.[55]

KIDNEY TRANSPLANTATION

Recurrence is observed in one-third of transplanted patients for MN, and the risk of recurrence is higher in PLA2R-positive patients.[61] When kidney transplantation is planned in MN patients, the relationship of MN with anti-PLA2R must be revealed. Patients with high anti-PLA2R levels (>45 RU/mL) have a 50% risk of MN recurrence in the graft. In patients whose anti-PLA2R level cannot be measured, a biopsy from the native kidneys to study the tissue anti-PLA2R level accordingly and calculate the risk of MN recurrence is an accepted method to predict the risk of recurrence.

In patients whose primary disease is MN and who underwent kidney transplantation, monthly proteinuria follow-up for the first 6-12 months after transplantation and kidney biopsy if proteinuria is detected above 1 g/day are recommended. In patients with positive anti-PLA2R levels, immunological

follow-up is recommended at intervals of 1-3 months in the first 6-12 months, and kidney biopsy is recommended if proteinuria is detected above 300 mg in patients with high or persistently high antibody levels. When MN recurrence is detected in the graft, it is recommended to start the RAS inhibitor at the maximum tolerated dose and to administer 2 doses of RTX therapy in patients with proteinuria above 1 g/day.[13,62] Although data on anti-THSD7A and kidney transplantation are insufficient, similar algorithms are likely to be used in the evaluation and follow-up of patients with anti-THSD7A-associated MN.

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