Membranous Nephropathy Post-Transplantation: An Update of the Pathophysiology and Management

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Abbreviations

ABMR, antibody-mediated rejection
AntiPLA2R, anti-phospholipase A2 receptor antibodies
AntiTHSD7A, anti-thrombospodin type-1 domain-containing 7A antibodies
dnMN, de novo membranous nephropathy
DSA, donor-specific antibodies
GBM, glomerular basement membrane
GWAS, genome wide association study
KT, kidney transplantation
MN, membranous nephropathy
PLA2R, phospholipase A2 receptor
pMN, primary membranous nephropathy
PTNS, post-transplant nephrotic syndrome
rMN, recurrent membranous nephropathy
THSD7A, thrombospodin type-1 domain-containing 7A
ABSTRACT

Membranous nephropathy (MN) is a common cause of nephrotic syndrome after transplantation and is associated with an increased risk of allograft loss. MN may occur either as a recurrent or as a de novo disease. As in native kidneys, the pathophysiology of the MN recurrence is in most cases associated with anti-phospholipid A2 receptor antibodies (antiPLA2R). However, the post-transplant course has some distinct features when compared to primary MN, including a lower chance of spontaneous remission and a greater requirement for adjuvant immunosuppressive therapy to induce complete remission. Whereas the efficacy of rituximab in primary MN is now well established, no randomized studies have assessed its effectiveness in MN after transplant, and there are no specific recommendations for the management of these patients. This review aims to synthesize and update the pathophysiology of post-transplant MN, as well as to address unsolved issues specific to transplantation, including the prognostic value of antiPLA2R, the risk of living-related donation, the link between de novo MN and rejection, and different therapeutic strategies so far deployed in post-transplant MN. Lastly, we propose a management algorithm for patients with MN who are planning to receive a kidney transplant, including pre-transplant considerations, post-transplant monitoring and the clinical approach after the diagnosis of recurrence.
**Introduction**

The development of proteinuria after kidney transplantation (KT) is a common occurrence, with 3% to 14% of recipients showing post-transplant nephrotic syndrome (PTNS)\(^1\)\(^-\)\(^5\). The risk of graft loss due to persistent proteinuria remains significant\(^6\),\(^7\), and has been reported in approximately 48-77%\(^2\),\(^4\),\(^6\),\(^8\),\(^9\) of PTNS cases, after five years of follow up.

PTNS is associated with several etiologic causes that frequently overlap\(^4\),\(^9\) (Figure 1). In particular, the recurrence of glomerulonephritis post-transplant has a worse prognosis\(^2\),\(^10\),\(^11\), depending on its nature and whether it remits or persists\(^4\). The reported risk of recurrence is very heterogeneous and depends mainly on the type of primary glomerular disease and other factors, such as surveillance biopsy policies or demographic characteristics\(^10\)-\(^12\). In fact, a reliable estimation of recurrent or \textit{de novo} glomerulonephritis in the kidney graft is challenging to obtain, since diagnostic criteria between transplantation centers can vary widely\(^13\). Nevertheless, even if underestimated, as is likely, recurrence seems to be responsible for about 15% of death-censored allograft losses, which would represent the third most frequent cause of allograft loss after 10 years\(^14\).

In native kidneys, membranous nephropathy (MN) is one of the most common causes of nephrotic syndrome in adults, with an estimated worldwide incidence of 1.2/100,000/year\(^15\). It is an antibody-mediated glomerular disease, defined by subepithelial immune deposits containing antigen, IgG, and complement components. While about 20% of MN cases are secondary to systemic conditions - such as infections, autoimmune diseases, cancer or medications - most others are considered primary (pMN). In the absence of specific treatment or in cases of partial remission despite treatment, pMN will progress to end-stage renal disease in approximately 25% of patients within 8 years, and 50% within 10–15 years\(^16\),\(^17\), and will entail a substantial risk of post-transplant recurrence\(^18\). MN may be identified in a graft, as a recurrence of the original disease (rMN) or as
a de novo MN (dnMN), and in exceptional cases, as a donor-derived disease. The rate of recurrence varies among studies, ranging from 7% to over 44% of patients. The rate of recurrence is higher and disease is detected earlier in transplant centers that perform surveillance biopsies, although rMN is less common than recurrent focal and segmental glomerulosclerosis or membranoproliferative glomerulonephritis. The recurrence is frequently related to the same autoantibody that caused the original disease on the native kidneys but, in this context, it is targeting the transplanted kidney (e.g., anti-PLA2R antibodies in PLA2R-associated rMN, anti-THSD7A in THSD7A-associated rMN). rMN can vary in severity from a subclinical finding on biopsy to proteinuria, PTNS and graft loss. By contrast, dnMN represents a separate entity with a different clinical course.

Currently, the pathophysiology and treatment of pMN are well established (in particular the use of rituximab). However, our understanding of MN recurrence after KT needs to be expanded in order to optimize its diagnosis and its management, and therefore, to improve long-term graft survival. To this end, this review aims to present the outstanding issues regarding MN after transplantation, as well as the treatment of the recurrence, for which only limited data is available. We will not discuss secondary MN and we will focus on pMN recurrence and dnMN.

1. Post-transplant membranous nephropathy pathophysiology: two different diseases?

MN is an antibody-mediated glomerular disease defined by subepithelial immune deposits containing antigen, IgG and complement components. Its pathogenesis has long been known to be caused by the binding of antibodies to podocyte antigens with the deposition of immune complexes. With the participation of complement activation, these subepithelial immune deposits lead to podocyte injury, with loss of slit diaphragm functionality and the development of
initial proteinuria\textsuperscript{29}. As the deposits accumulate, formation of extra basement membrane extensions (“spikes”) as well as around the deposits occur, followed by progression of proteinuria\textsuperscript{29}. While about 20\% of MN cases are secondary to systemic conditions such as infections, autoimmune diseases, cancer or medications, most others are considered primary, namely a glomerular-specific autoimmune disorder without an associated systemic condition. For this latter group (formerly known as idiopathic MN), the human target podocyte antigen remained elusive until 2009, when autoantibodies to the M-type phospholipase A2 receptor (PLA2R)\textsuperscript{30} was discovered. PLA2R is a trans-membrane protein, a member of the mannose receptor family and has at least three identified humoral epitopes to which PLA2R antibodies (antiPLA2R) bind\textsuperscript{31,32}. This finding has remarkably improved the understanding of the pathogenesis and the management of pMN\textsuperscript{33,34}. In native kidneys, around 70-80\% of pMN cases are mediated by circulating antiPLA2R\textsuperscript{31,35}, while approximately 3-5\% are caused by another target antigen in podocytes, the thrombospondin type-1 domain-containing 7A (THSD7A)\textsuperscript{36,37}. Both of these antibodies appear to be the cause of the autoimmune deposits, and they are usually not detected in secondary forms of MN. To note, positive antiPLA2R MN cases have been reported in patients with sarcoidosis\textsuperscript{38}, liver autoimmune disease\textsuperscript{39}, hepatitis B (up to 64\% of positive antiPLA2R cases in a Chinese cohort\textsuperscript{40}), and in exceptional cases of class V lupus nephritis\textsuperscript{41}. Complement is known to play a substantial role in the pathogenesis of pMN\textsuperscript{42}. The typical deposits activate the complement cascade locally and cause podocyte injury, and C4d and C3 staining are found within immune deposits in most pMN\textsuperscript{43-46} biopsies. However, C1q staining is typically absent in pMN\textsuperscript{47}. Thus, this complement activation likely operates through the mannose-binding lectin and/or the alternative pathway\textsuperscript{48}. 
As in native kidneys, the diagnosis of MN after transplant is confirmed by biopsy. The characteristic immune deposits are first seen by immunofluorescence microscopy as diffuse granular deposits of IgG along the capillary walls, and later by light microscopy as fucsinophilic subepithelial deposits located between basement membrane “spikes” on the external side of the glomerular basement membrane (GBM). In pMN, electron microscopy confirms the subepithelial localization of the electron-dense deposits and the extensive podocyte foot process effacement. In secondary MN, deposits can also be seen in the subendothelial or mesangial areas. In either case, the deposits and the subsequent matrix laid down cause progressive thickening of the GBM.

In the setting of KT, two main types of MN with distinct pathophysiologic mechanisms have been described. While the recurrence of pMN is related to the same glomerular-specific autoimmune disorder (the majority of which are due to antiPLA2R), de novo MN involves a different immunological mechanism.

In cases of recurrence, the disease represents either a persistence or a relapse of the original disease in the allograft. In the latter, circulating autoantibodies are absent at the time of transplant but may re-emerge years after transplant possibly due to weaning of immunosuppression and/or second hits such as infections. PLA2R antigens co-localize with antiPLA2R IgG (usually IgG4) and are seen by immunofluorescence microscopy or immunohistochemistry along the GBM (subepithelial deposits). These deposits may persist for weeks to months even after the circulating antibody is undetectable and new immune complex formation has ceased. In cases of complete remission, the deposits are gradually reabsorbed and eventually disappear. C4d staining is also often detected with a diffuse granular pattern, but this is not always the case. Similarly, C3 staining is not as prevalent in rMN as it is in native kidney disease. In the early phases of the disease, light microscopy abnormalities may also be missed and membrane spikes are not observed. Therefore,
electron microscopy is often necessary to confirm the diagnosis of rMN. It is important to note that some forms of secondary MN can also recur in the graft due to persistence of the underlying autoimmune disease, such as in membranous lupus nephritis\textsuperscript{55}.

In patients with dnMN, a well-defined pathogenic process has not been determined. Several factors have been proposed as risks for dnMN development, such as hepatitis B and C\textsuperscript{56-59}, post-transplant malignancy, renal infarction\textsuperscript{60}, ureteral obstruction\textsuperscript{61}, toxicity of anti-vascular endothelial growth factor therapy\textsuperscript{62}, and in exceptional cases complicated recurrent IgA nephritis\textsuperscript{63}. However, causality is difficult to prove. Some cases might be similar to secondary MN in native kidneys (e.g. hepatitis, neoplasia)\textsuperscript{64}, while others might be related to specific alloimmune responses to donor-specific antigens such as antibody-mediated rejection\textsuperscript{65,66}.

Regarding histology, some differences have been reported between dnMN and rMN. While IgG4 is the dominant or co-dominant IgG subclass within the glomerular deposits in rMN\textsuperscript{67} (as described in pMN), some authors have found IgG1 to be dominant or co-dominant in dnMN forms\textsuperscript{68,69}.

Secondly, dnMN is less often associated with antiPLA2R\textsuperscript{24,70,71}. Debiec et al. detected antiPLA2R in none of the sera of nine patients with dnMN, nor in their biopsies, whereas 50\% of rMN patients showed PLA2R positive staining\textsuperscript{24}. Larsen et al. found positive PLA2R staining in 10 of 11 biopsies from patients with rMN, whereas only 1 of 11 patients with dnMN showed positive PLA2R in the biopsy\textsuperscript{70}. Finally, Ward et al. demonstrated circulating immune complexes in the sera of 7 patients with dnMN, and in 5 of them, the IgG was targeted against brush border or tubular epithelial or interstitial antigens\textsuperscript{72}, instead of podocyte antigens as seen in pMN\textsuperscript{34}.

Lastly, dnMN can present with some mesangial hypercellularity, a focal and segmental distribution of the deposits\textsuperscript{73}, and with heterogeneous stages of the deposits in the same biopsy\textsuperscript{74}, findings that are not typically described in rMN and pMN.
Hence, dnMN appears to have a different immunological pathogenic mechanism. An initial trigger such as viral infection, urinary obstruction, ischemia, or an immunological process, may lead to podocyte damage. If that trigger is severe or persists in time, cryptic podocyte protein antigens (different from those involved in primary MN, eg. PLA2R) may be exposed and drive an immune response with new antibody generation\textsuperscript{64}. This phenomenon contributes to the formation of in situ antigen-antibody immune-complexes and the typical subepithelial deposits of MN. As suggested by Couser et al., the immune response might be different depending on the antigen’s nature and triggers, which would explain the different histological and clinical patterns between rMN and dnMN\textsuperscript{31}. Figure 2 presents the two main pathogenic mechanisms of post-transplant MN.

2. Can we predict which patients with pMN are at higher risk of recurrence post-transplant?  
And is it possible to know which ones are going to have a worse prognosis once recurrence has occurred?

The severity of recurrence varies, from a subclinical histology feature to proteinuria and PTNS, but in either case, rMN has been shown to be a risk for graft failure\textsuperscript{10-12,25,75}. For example, in two recent large studies, when pMN patients were compared to patients without the possibility of recurrent disease (polycystic kidney disease), Pippias et al. found an adjusted relative risk of death-censored graft failure at 10 years of 1.60 in 708 patients with pMN\textsuperscript{76}, and Pruthi et al. found a risk of 1.99 in 183 patients with MN\textsuperscript{77}. This higher risk appears to be attributable to recurrence\textsuperscript{10,76,77}. Therefore, knowing which patients with pMN are at high risk of recurrence is of vital importance to implement early diagnostic strategies and appropriate prophylactic treatment if needed.

It is important to note that the recurrence is detected earlier and more frequently in transplant centers that perform surveillance biopsy protocols\textsuperscript{22,7124}. Interestingly, some cases are diagnosed
as early as 6 days after transplantation\textsuperscript{13,78}. Without a systematic biopsy protocol, the disease usually manifests clinically 1 to 3 years after transplantation\textsuperscript{79}. However, even with protocol biopsies, a second group of recurrences may occur years post-transplantation. This later presentation could be related to patients that had no circulating autoantibodies at time of transplant and developed a relapse once immunosuppression is weaned post-transplantation or when a secondary hit, such as infection, triggered re-emergence of the auto-antibodies\textsuperscript{13}.

The risk factors for recurrence in MN remain elusive as documented by the existing literature\textsuperscript{20}. Very often, contradictory results are found in different studies, mainly due to the retrospective nature of the studies and the small number of study subjects\textsuperscript{74,80,81}. The following factors have been associated with a higher risk of recurrence: high levels of proteinuria pre-transplant\textsuperscript{82}, shorter waitlist times\textsuperscript{81} and antiPLA2R titer\textsuperscript{82}. All these factors still need to be confirmed by further studies. Two particularly relevant factors have been covered in a specific section of this review: antiPLA2R titer at the time of transplantation\textsuperscript{82-85}, and the potential risk associated with related-living donor KT.

During the post-transplant follow up, and in contrast to pMN, clinical and/or histological features do not seem to impact the prognosis in rMN. Grupper et al. showed that spontaneous clinical remission occurred in almost one-third of patients in their cohort, surprisingly similar to pMN remission rates, and was associated with histologic resolution of the injuries\textsuperscript{82}. The remaining patients progressed and, regardless of the initial type of presentation, had increased risk of graft failure. Even with low amounts of proteinuria, the disease can progress quickly\textsuperscript{82,83,85}. Histological features of rMN at diagnosis are also not relevant to predict the prognosis. In particular, the persistence of foot process effacement has been shown despite clinical remission. However, the persistence of antiPLA2R at high levels after transplant does seem to reflect a progressive disease, as evidenced by serial biopsies in the same patient\textsuperscript{51}. Finally, whereas several factors such as
regulatory T cells may be useful in predicting the response to rituximab in pMN, no factors have yet been connected with rituximab response in rMN\textsuperscript{86}. Patients with high antiPLA2R levels or severe proteinuria in the pre-transplant period should be closely monitored after transplant and a possible prophylactic therapy such as rituximab in case of living donation should be considered. In the post-transplant period, a careful monitoring of proteinuria and antiPLA2R titers, as well as surveillance biopsies would allow an earlier diagnosis of rMN, timely treatment (without a period of observation) and better outcomes\textsuperscript{23,82}. An algorithm of management is proposed in Figure 3.

3. What is the role of PLA2R and antiPLA2R antibodies in the detection/prevention of MN recurrence?

The discovery of antiPLA2R in the pathogenesis of almost 80\% of pMN cases\textsuperscript{31,33,34} has spurred an important change in the evaluation of these patients. In particular, the treatment is now adjusted to account for the presence and levels of the pathogenic antibody, which are both linked to disease activity\textsuperscript{87,88}. Baseline titers have been shown to correlate with disease activity, the probability of achieving spontaneous remission\textsuperscript{89}, the response to treatment with rituximab, and long-term prognosis\textsuperscript{87}, with falling titers indicating immunologic remission and, eventually, clinical remission. Histologically, the characteristic subepithelial deposits, combining IgG and antiPLA2R, persist for months to years after the serum antibody is undetectable.

In the setting of MN after transplantation, three different entities may be described: PLA2R-associated rMN, non-PLA2R-associated rMN (which could include THSD7A-associated rMN or other yet-to-be-discovered antigens), and de novo MN. The first question is whether antiPLA2R
levels might have a predictive value in rMN after positive antiPLA2R pMN. In 2010, Stahl et al. suggested for the first time through a single case report that rMN may be caused by circulating antiPLA2R that binds to an antigen expressed on the donor’s podocytes. Therefore, antiPLA2R would be a risk factor for recurrence at the time of transplantation. However, in another study with 10 patients with rMN and 9 with dnMN, Debiec et al. observed that only 5/10 patients with positive antiPLA2R at the time of transplantation had a recurrence later on, with an increase of antiPLA2R titers but without a significant correlation between recurrence onset and antibody kinetics, in contrast to what is observed in pMN. Additionally, none of the dnMN patients had antiPLA2R. After investigating 6 control patients with antiPLA2R pMN in their native kidneys but who did not experience recurrence within a 2-year follow-up period, 3/6 had high antiPLA2R levels at the time of transplantation. Seitz-Polsky et al. studied 10 patients with primary MN and positive titers of antiPLA2R at the time of transplantation and showed that the persistence of antiPLA2R after 6 months post-transplantation was a risk factor for recurrence. This was confirmed by two other studies. Notably, the last study by the Mayo Clinic with the largest cohort of patients with rMN (n=33), found that patients with antiPLA2R at the time of transplant had a higher risk of recurrence. Interestingly, four patients without detectable antiPLA2R at the time of transplantation also experienced recurrence, which may be interpreted as a relapse of the disease with re-appearance of the anti-PLA2R antibodies years later. Antigen cross-reactivity in the setting of infection or reduction of immunosuppression over time may allow re-emergence of anti-PLA2R antibodies. Lastly, some patients may be “resistant” to the standard immunosuppression post-transplant. Overall, rMN involves a complex interplay between donor-recipient that is still poorly understood but include HLA differences, possible variants in the PLA2R antigen in the donor, effect of immunosuppression or/and secondary hits such as infection, rejection or ischemia-reperfusion injury post-transplantation.
There is a clear evidence of genetic predisposition in the development of pMN. Stanescu et al. performed the first genome wide association study (GWAS) in three Caucasian populations with pMN\textsuperscript{91}. They identified two distinct risk loci highly associated with pMN: the HLA-DQA1 gene and the M-type phospholipase 2 receptor gene. Two more recent GWAS studies in Chinese patients with pMN have identified other independent HLA risk alleles including DQA1*05:01, DRB1*15:01/DRB1*03:01\textsuperscript{92} and DRB3*02:02\textsuperscript{93}. To note, DQA1*05:01 had already been linked to a high level of antiPLA2R antibodies in 2013\textsuperscript{94}. They are not specific to pMN and have already been identified in the general population as being related to autoimmune predisposition. All these studies show that homozygosity for these high-risk alleles, in both HLA and PLA2R genes, increased the relative risk of pMN almost 80 times in Caucasian patients and 10 times in Chinese patients. This suggests an interaction between pathological HLA class II alleles and PLA2R variants, consistent with a genetic susceptibility to develop pMN. However, these polymorphisms within HLA risk alleles and in PLA2R epitopes have not yet been well evaluated in rMN.

Couchoud et al. found that the HLA-DR3 allele was higher in patients who had primary MN and post-transplant recurrence than in those without recurrence\textsuperscript{21}. Quintana et al. showed that 6 of 7 patients with positive antiPLA2R rMN were carriers of the HLA risk allele DQA1*05:01/05, which was significantly associated with higher pre-transplant antiPLA2R levels\textsuperscript{90}. Wetzels and Andrésdóttir found that HLA-A3 was carried by 75% of donors with recurrence compared to 15% of donors without recurrence; although the mechanism is unclear, molecular mimicry could play a role\textsuperscript{95}. A sequence analysis of epitopes in the different risk-HLA antigens and PLA2R might help to determine whether cross-reactivity is a potential explanation for these correlations.

To conclude, the presence of antiPLA2R antibody in the pre-transplant period or at the time of transplantation appears to have clinical utility as a predicting risk factor for rMN\textsuperscript{85} and requires close serologic monitoring. This should also be done in patients without antiPLA2R at the time of
transplantation, but who were previously positive or tissue antigen positive (or of unknown status) on a native kidney biopsy. In either case, the appearance, persistence or increase of antibodies should prompt a biopsy, as well as any case with significant proteinuria regardless of antiPLA2R status. PLA2R tissue staining might be more sensitive than serological testing of antiPLA2R, in particular for the differentiation between de novo MN and rMN when the native kidney disease is unknown. Lastly, antibody-mediated podocyte injury depends on a number of factors other than antibody titers, including amount of antigen expressed, affinity of the antibody, capacity to activate complement and to bind to different Fc receptors according to glycosylation pattern. Therefore, a more detailed characterization of antiPLA2R antibodies may be required to improve its specificity in predicting disease recurrence.

4. Is recurrence risk increased with living related donor KT?

Prior to the discovery of antiPLA2R, living-donor KT was considered to be one of the main pre-transplant risk factors for pMN recurrence. As we discussed above, MN is partly related to autoimmune pathophysiology, within a context of genetic predisposition. PLA2R and HLA risk alleles have been associated with a relative risk for primary MN. Therefore, receiving a kidney from a living related donor who might share susceptibility factors might increase recurrence risk. Andrésdóttir and Wetzels hypothesized that recurrence would occur only if recipient and donor share the same target antigen polymorphism, such as PLA2R, which would be more common in the case of genetically related donors. The same could apply for HLA risk alleles both in living and deceased KT. In their cohort of 23 adult Caucasian patients with pMN who received a KT between 1970 and 2005 with a mean follow-up of 7.4 years, 8 patients (34.8%) at an average of 12 months after transplantation had a biopsy-proven recurrence. The cumulative recurrence rate at 3 years was 70% in living related donors (4
siblings, 1 parent) and 21% in unrelated donors. However, PLA2R gene sequencing of donors and recipients with MN$^{24}$ would be necessary in order to prove this hypothesis, although it has not been evaluated so far.

To our knowledge, no prospective study has described specifically the impact of living related donation on rMN. Two recent analyses have highlighted another perspective. First, Pippias et al. analyzed data from the European Renal Association-European Dialysis and Transplant Association Registry and showed a significant superior allograft survival with living donors compared to deceased donors in pMN, independently of donor-recipient relation$^{76}$. Kennard et al. investigated the effect of living related donation on rates of glomerular disease recurrence and graft outcomes from 28 years’ of Australia and New Zealand Dialysis and Transplant Registry data$^{98}$. Among patients with pMN, there was no difference in the rate of recurrence-free survival based on the 3 donor categories, related living, unrelated living or deceased donors.

In conclusion, genetic factors, such as PLA2R or HLA polymorphism, might contribute to rMN, and this genetic susceptibility could enhance the risk of recurrence in the case of living related donors. However, the increased recurrence risk seems not to outweigh the superior outcomes typically associated with living donation compared to deceased. So far, living donation, related or not related, remains the best choice for pMN recipients. If more than one donor is available, we anticipate that incorporating genetic assessment for PLA2R high-risk variants may help with living donor selection, favoring patients with absent PLA2R high-risk variants. Though further data is needed to elucidate the contributions of donor and recipient genetic susceptibilities.

5. De novo membranous nephropathy and antibody-mediated rejection: myth or reality?
Although the typical disease described after transplant is recurrence of pMN, we can also find cases not related to the native kidney disease and, therefore, *de novo* MN. dnMN has been scarcely described in the literature, even though it might occur with equal frequency to rMN in the allograft. It is still considered the most common form of *de novo* glomerulonephritis, with a reported incidence around 1-2% in adult transplant recipients and up to 9% in pediatric patients. The clinical presentation of dnMN may vary from asymptomatic proteinuria with an indolent clinical course to (less frequently) an accelerated evolution leading to graft loss, usually appearing later in the post-transplant period compared to rMN.

The possible triggers of this disease are not clearly defined and likely multifactorial. In particular, it has been known for almost 30 years, that dnMN is accompanied by histological features of rejection. In 1989 Truong et al. described the first significant case series supporting the association between mixed rejection and dnMN. Among 10 dnMN cases, 6 had biopsy-proven acute cellular rejection before the dnMN diagnosis, 8 had concomitant inflammatory cells in the glomeruli, and all cases presented with signs of possible rejection, such as vascular changes, interstitial inflammation, tubulitis, and accumulation of subendothelial electron-lucent material by electron microscopy. In the same paper, the authors also reviewed 95 cases of dnMN published before 1989, with 96% of patients with histological findings of cellular rejection and more of 50% with concomitant signs of humoral rejection. In 1993, Monga et al. found that one-third of dnMN cases had signs of active glomerulitis, supporting the notion of a concomitant humoral rejection. Schwartz et al. reported that 17 of 21 dnMN patients showed signs of vascular rejection. Subsequently, several findings related to antibody-mediated have been observed in cases of dnMN, such as peritubular capillaritis and C4d deposition. Since many of these studies were performed before the routine use of anti-HLA testing, it is highly possible that chronic ABMR may account for some of these *de novo* membranous cases.
Interestingly, El Kossi described a kidney transplant recipient who developed clinically-significant dnMN associated with typical signs of antibody-mediated damage, in the context of a HLA-DQ7 DSA\textsuperscript{102}. Surprisingly, in parallel with decreasing DSA titer, he experienced a complete remission after 18 months of follow up. More recently, Wen J et al. compared the histological features of dnMN and pMN\textsuperscript{69}. The authors demonstrated that dnMN cases presented with higher interstitial inflammation, peritubular capillaritis and C4d deposition, compared to pMN. Notably, PLA2R antigen staining was negative in all dnMN cases.

One peculiar case of secondary MN is the one associated with graft-versus-host disease, which often occurs after hematopoietic cell transplantation\textsuperscript{103}. In this disease, the transplanted donor leukocytes attack the recipient’s tissue, in particular endothelial cells. This has been described as a manifestation of an alloimmune response primarily involving T cells without clear humoral component (C4d staining negative). Steroids treatment are frequently effective, leading to complete or partial response\textsuperscript{103}. Nevertheless, histological rejection features have also been proven to be absent in some cases reported in the literature\textsuperscript{70}.

These data suggest that dnMN, in a majority of cases, is a manifestation of an unique form of humoral alloreactivity, which may involve an alloimmune response directed against podocytes. Whether this is the result of antibody reactivity to HLA class II antigens expressed on podocytes or other podocyte planted-antigens will require further investigation since the presence of subepithelial deposits imply an in situ immune complex formation.

6. Treatment: differences between pMN and evidence with rituximab
Whereas pMN treatment trials are pointing towards rituximab as the first line, data about rMN treatment is limited and composed mainly of case series and small sample-size retrospective studies, without any randomized controlled trials.

In the setting of rMN, all patients should be treated with the standard supportive treatment of any glomerulopathy from the time of diagnosis, including renin-angiotensin system blockade, strict blood pressure control and symptomatic treatment with diuretics, statin therapy and anticoagulation if needed. To note, clinicians should carefully monitor serum potassium, creatinine and hemoglobin levels after initiation of renin-angiotensin system blockade, in particular in the early post-transplant period. Worsen hyperkalemia, significant rise in creatinine and drop in hemoglobin are potential side effects of ACEI/ARB when used in combination with immunosuppressive drugs such as tacrolimus and mycophenolate. Despite supporting treatment, most rMN patients progress\textsuperscript{20}. As we described above, relevant prognostic factors of severity are lacking. rMN patients appear to reach end-stage renal disease more often than pMN patients do, even in cases of non-aggressive disease at diagnosis. Therefore, a first period of observation recommended for pMN, might be harmful in rMN. Therefore, all patients should be considered for intensive treatment with immunosuppressive therapy if significant proteinuria is present (>1g/day).

In contrast to pMN, there is no evidence that steroids, alkylating agents, calcineurin inhibitors or mycophenolic acid provide specific benefits in the setting of rMN\textsuperscript{100}, in particular since most transplant recipients who experience a recurrence are already receiving one or more of these agents. Nonetheless, it is possible that, in selected cases, the standard transplant immunosuppressive regimen (induction and maintenance) might be sufficient to induce immunological remission\textsuperscript{20,82}. 
The discovery of antiPLA2R and antiTHSD7A provides a clear pathophysiological basis for promoting therapies that target B cells, to prevent antibody production and subsequent immune deposition. Rituximab, a monoclonal antibody targeted against CD20 expressed by B-lymphocytes, is known to be a safe and efficient treatment for pMN. Its efficacy in pMN, as well as the confirmation that most rMN cases are mediated by the same autoimmune “B cell-mediated” process as pMN, have led to rituximab being used in rMN treatment. Table 1 and 2 summarize the case series and observational studies that describe rMN cases and outcomes with different treatment regimens.

Overall, promising results with higher rates of clinical remission have been seen with rituximab. Rituximab was first successfully used for rMN in 2006. After that, several case reports and retrospective studies have reported their experience. The largest cohort comes from the experience of Mayo Clinic, whose protocol biopsies (4, 12, 24, and 60 months post transplant) allow early diagnosis and the assessment of histologic changes during the course of the disease. As shown in these serial protocol biopsies, rituximab appears to result in complete histological remission, with the disappearance of dense deposits, the improvement of foot process damage, and the regression of IgG and C3 deposition. In 2016, the Mayo Clinic reported 63 patients with pMN who underwent a KT. 48% of these had histological recurrence, but only about 50% of these presented with clinical manifestations. Rituximab was given to patients with more than 1g/g of proteinuria and showed a high rate of partial or complete clinical remission (82%), with 40% showing histological remission of the disease and no relapse in a median follow-up time of 15 months. Interestingly, the rate of complete remission and the B-cell depletion induced by rituximab appear to be higher and to last longer in the graft than in native kidneys. One possible explanation is the potential additive role of T cell-targeted therapies, which transplant patients already receive, in increasing the remission rates. The response to rituximab does not
correlate with the level of proteinuria at the time of treatment\textsuperscript{20,23}. However, the closely monitoring of proteinuria post-transplant may earlier detect rMN, creating a timing bias of diagnosis and treatment compared to pMN.

As for rituximab dosage, the data is varied but, in general, an initial infusion of 1 g is recommended, followed by a second infusion 2 weeks later. Taking into consideration the potential for over-immunosuppression in transplant recipients who are already undergoing maintenance immunosuppressive therapy, rituximab dosage should be individualized in recipients with a high risk of infection\textsuperscript{111}. The follow-up of these studies was fairly short and side effects of rituximab, such as infections or the long-term risk of malignancy, have not been evaluated.

Despite achieving complete depletion of circulating B cells with rituximab, some patients do not achieve complete remission both in pMN\textsuperscript{33,107,112} and rMN\textsuperscript{82}. Some authors point to the key role of long-lived memory plasma cells (CD19\textsuperscript{−}CD20\textsuperscript{−}CD38\textsuperscript{+}) in this rituximab-resistance\textsuperscript{109,113} since these cells are known to produce considerable amounts of IgG auto and alloantibodies\textsuperscript{114}, and lack rituximab CD20 target. Therefore, the use of plasma-cell-depleting therapy has emerged in recent years. Barbari et al. reported the first successful case of rMN treated with bortezomib\textsuperscript{115}. In this setting, other therapies such as anti-CD38 monoclonal antibodies might be therapeutic options for rMNs. Immunologic biomarkers for T and B cell profiles may help to identify different subsets of patients that are most likely to respond to these therapies\textsuperscript{116}.

In conclusion, rMN has better prognosis if treatment is started early. Rituximab might be safe and effective for inducing remission. However, in cases of subclinical rMN, a balance between early treatment and the risk of infection should be considered. Moreover, the effectiveness of rituximab versus other therapies has not yet been assessed. Figure 3 presents an algorithm for pre-transplant assessment, follow-up and treatment of patients with rMN.
Conclusions

Although the pathophysiology of primary MN is better understood and the outcomes of primary MN have improved, MN after renal transplantation remains a less well-understood disease and a significant cause of graft failure. Knowing the differences between recurrent and de novo MN is key to establishing surveillance strategies for these patients. Monitoring sera antiPLA2R (or antiTHSD7A) and early graft biopsies in patients with primary MN who undergo a KT appears to be crucial for a prompt diagnosis and adequate treatment. Rituximab emerges as a valuable therapeutic approach but more studies are needed to determine its true long-term efficacy and safety profile when combined with maintenance immunosuppression.

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Figure legends

Figure 1. Work-up and differential diagnosis of proteinuria after transplantation

Abbreviations: CNI, calcineurin inhibitor; FSGS, focal segmental glomerulosclerosis; MN, membranous nephropathy; MPGN, membranoproliferative glomerulonephritis; mTOR, mammalian target of rapamycin

Figure 2. Pathophysiology of post-transplant membranous nephropathy

Legend: Recurrent membranous nephropathy (rMN) and de novo membranous nephropathy (dnMN) lead to the same injuries, related to the formation of subepithelial immune complexes. However, the composition and the triggers of these immune complexes are different: mainly IgG4 against PLA2R1 antigen in rMN; IgG1 against hidden antigens in dnMN with various triggers including rejection. A, IgG staining (green) along glomerular capillaries in a subepithelial distribution in a patient with rMN (immunofluorescence, original magnification x400). B, Positive PLA2R staining (green) along glomerular capillaries in a patient with rMN (immunofluorescence, original magnification x400). C, Ultrastructural appearance of rMN. Note the subepithelial electron dense deposits (red arrows) that are associated with extensive foot process effacement (electron microscopy, original magnification x10,000) D, Light microscopic appearance of rMN. Note the lack of well developed “spikes” (Jones methenamine silver, original magnification x600) E, Diffuse C4d staining (green) along peritubular capillaries in a patient with dnMN and concurrent antibody-mediated rejection (immunofluorescence, original magnification x200)

Abbreviations: antiPLA2R, anti-phospholipase A2 receptor antibodies; GBM, glomerular basement membrane; Ig, immunoglobulin
Figure 3. Algorithm for the pre-transplant assessment, post-transplant screening and management of recurrent membranous nephropathy post-transplant.

Abbreviations: ACEi, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; Anti-PLA2R, anti-phospholipase A2 receptor antibodies; D, day; Ig, Immunoglobulin; PLA2-R, phospholipase A2 receptor; MN, membranous nephropathy