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New insights into immune mechanisms in idiopathic membranous nephropathy

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New insights into immune mechanisms in idiopathic membranous nephropathy

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New insights into immune mechanisms in idiopathic membranous nephropathy

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Abstract

Membranous nephropathy (NM) is recognized as the most common cause of nephrotic syndrome in adults. Idiopathic NM (iMN) behaves as an autoimmune glomerular disease with three circulating autoantibodies specific for native podocyte antigens identified by now, which has transformed the management and monitoring of iMN. The autoimmune character of iMN was described in 2009 when PLA2R1 was identified as a major target antigen in more than 70% of cases of iMN without apparent secondary causes, particularly in men. PLA2R1 is commonly tested in cases of iMN by serologic tests for anti-PLA2R1, and in kidney biopsy by staining for PLA2R1, with more than 99% specificity and 78% sensitivity for the diagnosis of iMN. THSD7A was the second antigen recognized, and added to the panel of iMN antigens. Recently, NELL-1 was identified and is expected to be a distinct biopsy marker for iMN. Measuring antibodies titers is of crucial importance since they have been proposed as biomarkers of MN autoimmune activity.

In this review, we describe recent advances of autoantibodies related to iMN, namely the anti-PLA2R1 and the anti-THSD7A, and its potential contribution as disease activity biomarkers, therapeutic monitoring, and outcome predictors.

Keywords: Glomerulonephritis, membranous nephropathy, nephrotic syndrome.

Introduction

Membranous nephropathy (NM) is recognized as the most common cause of nephrotic syndrome in Caucasian adults (approximately 25% of cases) and one of the leading causes of end-stage renal disease, as 30% of MN patients will evolve to this stage.[1-3] The mean age of diagnose is between 50 and 60 years old and affects mostly men (2:1).[1,4,5] The prevalence of MN varies strongly between geographic regions, affecting China on a greater scale.[1,2]

MN is not preceded by prodromal manifestations. In fact, the most prominent feature is the nephrotic syndrome, characterized by proteinuria (above 3.5 g/24h), hypoalbuminemia, various degrees of edema and elevated serum lipids, with normal or lightly altered kidney function. An underestimation of the incidence ought to be considered since that proteinuria below 2.0 g/24h without nephrotic syndrome has been described in 10% to 20% of cases.[1]

Idiopathic NM (iMN) is conceptually a single organ-specific autoimmune glomerular disease, in which circulating autoantibodies bind to an autoantigen on the surface of the podocytes, such as Neutral Endopeptidase (NEP)[6], Phospholipase A2 receptor (PLA2R1)[7], Thrombospondin type 1 domain-containing 7A (THSD7A)[8] and the recently discovered Neural epidermal growth factor-like1 protein (NELL-1).[9]

The landmark discovery of circulating antibodies specific for native podocyte antigens, particularly PLA2R1 and THSD7A, has transformed the management and monitoring of iMN, besides its contribution for diagnosis assessment.[1,2] Anti-PLA2R1 is present in 70-80% of patients with iMN and anti-THSD7A in only 2-3% of patients.[7,10,11] In the remaining cases, the target antigen is unknown, albeit a recent evidence of an additional autoantibody towards NELL-1 (Table1).[9]

Japan is an exception for this evidence, since the prevalence of anti-PLA2R antibodies in iMN is much lower than in other Asian Countries (about 50% of all patients with iMN).[2]

The kidney biopsy is the gold standard in recognizing the pattern of injury lesion of MN and remains the standard of care in most centers.[4] The characteristic features of histological examination of kidney samples includes thickening of the glomerular basement membrane on light microscopy with “spikes” on silver stain, corresponding to granular deposits by immunofluorescence of immunoglobulins (mainly IgG4) and complement proteins (C3) on the capillary walls. Glomerular IgG4 staining is associated with anti-PLA2R1 deposition in iMN.[12] Electron microscopy examination provides further detail, localizing the immune deposits at the subepithelial space, with thickening appearance at latter phases.[4,10,13] Immunofluorescence microscopy in anti-PLA2R1/THSD7A patients normally shows diffuse, uniform, finely granular deposits of IgG4 along outer surfaces of all capillary walls. Electron microscopy in iMN confirms exclusively subepithelial localization of electron-dense deposits. Any additional biopsy findings from the mentioned above, should prompt careful search for secondary causes.[4] The timing of the renal biopsy matters as the histology changes according to the onset of MN and reflects the stage of the.[2]

Secondary MN constitutes about 20% of all the cases and is associated with the presence of immune complexes possibly containing foreign antigens. It occurs in the context of infections (viral hepatitis B, viral hepatitis C, human immunodeficiency virus, syphilis, schistosomiasis), malignancy (solid tumors as lung, prostate, colon, and hematologic ones), autoimmune diseases (Systemic Lupus Erythematosus (SLE), thyroiditis), alloimmune diseases (graft versus host disease, autologous stem cell transplants), drugs (gold salts, penicillamine and nonsteroidal anti-inflammatory drugs) and environmental air pollution (China).[2-4] Furthermore, secondary MN should be suspected when the biopsy demonstrates mesangial or endocapillary proliferation on light microscopy, full house immunofluorescence or when the electron microscopy also show subendothelial, mesangial, or tubular basement membrane electron dense deposits, or even endothelial tubuloreticular inclusions.[14] IgG subclass staining can help with differentiating primary from secondary MN, because there is a preponderance

of IgG1 and IgG4 in primary MN; IgG1, IgG2 and IgG3 in lupus nephritis; and IgG1 and IgG2 in malignancy-associated MN.[14,15]

In this review, we describe recent advances of autoantibodies related to iMN, namely the anti-PLA2R1 and the anti-THSD7A, and its potential contribution as disease activity biomarkers, therapeutic monitoring, and outcome predictors.

Methods

The articles in which this review is based were found in MEDLINE data basis and Scopus data basis from January 2019 until December 2019 using keywords such as "Idiopathic Membranous Nephropathy", "Phospholipase A2 receptor 1", "Thrombospondin type 1 domain-containing 7A", "Neural epidermal growth factor-like1 protein". It was given preference to recent articles, preferably from the year 2018 and 2019 and were chosen review articles, editorial comments, randomized controlled trials and Kidney Disease Improving Global Outcomes recommendations.

Autoimmune nature of iMN

Much of what we know about the pathogenesis of MN derives from observations in the experimental rat model of Heymann nephritis (HN).[3] One of which was the influence of the complement in inducing proteinuria in experimental HN mouse models: after the binding of the auto-antibody to the podocyte antigen, an immune complex is formed and this triggers the activation of the complement system through the membrane attack complex C5b-9, thus causing cytotoxicity, destruction of filtration barrier and proteinuria.[4,16]

The complement has a role in the disease's progression to renal insufficiency in patients with MN. Indeed C5b-9 blocks podocyte autophagy by inhibition of lysosomal degradation of autophagosomes which increases podocyte apoptosis in human MN and

cultured podocytes. Inhibition of the podocyte autophagosomal/lysosomal system and ubiquitin proteasome system induces podocyte injury and worse proteinuria.[2]

However how anti-PLA2R1 and anti-THSD7A autoantibodies interact with the complement system is still a loose end and understanding it would clarify the mechanisms driving variable levels of proteinuria and clinical outcomes in patient subgroups.[1]

In the past decade, specific proteins have been identified as target antigens in human iMN and represent both intrinsic and planted antigens. The first demonstration that circulating antibodies could target an intrinsic podocyte antigen was identified in a rare case of antenatal MN caused by fetomaternal alloimmunization to neutral endopeptidase (NEP).[6] The mother of the affected child was genetically deficient in NEP and had been alloimmunized during a previous miscarried pregnancy. In the subsequent pregnancy, transplacental passage of anti-NEP antibodies led to in situ antigen-antibody complex formation and complement-mediated podocyte injury in the fetal kidney as a result of alloantibody binding to NEP expressed on the podocytes. Several more cases were subsequently identified, all due to truncating mutations in the maternal gene for NEP.[17]

In addition, a modified, cationic form of bovine serum albumin, derived from dietary sources and absorbed by the immature intestinal tract of infants, was found to behave as a planted antigen in rare cases of early childhood MN.[18]

The autoimmune character of iMN was described in 2009, when PLA2R1 was identified as a major target antigen in more than 70% of cases of iMN, particulary in men, without apparent secondary causes.[7]

PLA2R1 is a podocyte receptor and a transmembrane protein.[1,4] PLA2R1 is part of the mannose receptor structural family, which consists of the mannose receptor, DEC205, Endo180, FcRY and PLA2R1.[1,4,19] The latter belongs to a family of lipolytic enzymes involved in a potent proinflammatory host defense, phospholipid digestion and remodeling of cell membranes.[2,19,20] PLA2R1 is also involved in cell senescence

(apoptosis) and has been detected by immunofluorescence microscopy with monospecific anti-PLA2R1 antibody in human glomeruli, specifically, in podocytes.[2,7]

Anti-PLA2R1, mainly a IgG4, was detected circulating in serum by Western blot using human glomerular extracts as antigens, and in human MN sera as primary antibodies.[5,7] Mass spectrometry analysis provided further the identification of PLA2R1 as the target antigen.[1]

PLA2R1 compromises ten distinct globular domains, including a cysteine-rich (Ricin B) domain (Cys-R), a fibronectin II domain, and eight distinct C-type lectin domains (CDLD 1-8).[1,4,21,22] An immunodominant epitope, recognized by the anti-PLA2R1, was first identified in the N-terminal ricin domain.[1,21] Epitope spreading is the phenomenon responsible for the primary immunopathogenic event of autoimmune disease, in which other non-cross-reactive epitopes of the same protein are recognized by T or B cells and become new immunogenic sites.[1,4,21] This process is described in autoimmune diseases such as Pemphigus vulgaris, Human antiglomerular basement membrane disease and Multiple Sclerosis.[23] In iMN, it usually begins with the Cys-R, which is recognized by the antibodies and consequently spreads to other domains as CTLD1 and CTLD7 for PLA2R1.[1,4,21] Moreover, intramolecular spreading is thought to modulate remission and relapse in iMN.[23] *Seitz-Polski et. Al* showed that patients with anti-CysR-restricted activity were younger, had lower proteinuria and exhibited a higher rate of spontaneous remission and a lower rate of renal failure progression. By opposition, high anti-PLA2R1 activity and antibodies targeting 2 or 3 target epitope domains may be less likely to establish a spontaneous remission, being independent risk factors for poor renal prognosis.[23,24] Furthermore, this study showed that epitope profiles can shift during follow-up, which is associated with low remission rates.[21,23]

Anti-PLA2R1 binds to the ricin epitope with very high affinity and this has possible implications for the clinical interpretation of anti-PLA2R1 positivity. Low auto-antibody patient producers may seem to be seronegative until the antibody has saturated all the PLA2R1 epitopes on podocytes and only then become seropositive – the kidney acts as

a “sink”, absorbing all detectable circulating anti-PLA2R1. This means that PLA2R1 positivity accounts for 80-90% of cases, including the cases with low or no seropositivity.[1,25]

Subsequently, in 2014, another antigen was discovered, THSD7A, accounting 2-3% of iMN cases, with a higher prevalence on women.[1,8] This is a transmembrane protein composed of a large extracellular N-terminal region, a single pass transmembrane domain and a short intracellular C-terminal tail.[8] Histologically, THSD7A is found within GBM, with a typical linear pattern, more intensely than PLA2R1.[2,26] The clinical usefulness of anti-THSD7A measurement is currently under investigation.[27] In fact, the dominant epitope is THSD7A-associated MN is located within the most N-terminal part of the antigen, as happens in PLA2R1. Conversely, the reactivity in the C-terminal part is distributed over several epitope domains. Furthermore, serum from THSD7A-associated MN recognize more than one antigen domain and epitope profiles vary between the different patients, which is an important difference to PLA2R1, that has only three epitopes regions identified so far.[23,28] Noteworthy, when comparing with THSD7A-associated MN patients whose serum only recognized one or two epitope domains, patients whose serum identifies more than two epitopes domains had higher anti-THSD7A antibody levels, presented higher levels of proteinuria and reached a remission of proteinuria less often. In addition, during follow-up, two events were observed simultaneously: loss of recognition of one or more epitopes, decrease in anti-THSD7A antibody levels, and in most patients, remission of proteinuria.[29] As seen for anti-PLA2R1, a decrease in anti-THSD7A precedes a reduction of clinical disease activity.[8] Large cohort studies are needed to better understand the correlation between epitope spreading in THSD7A-associated MN at diagnosis, and the disease activity and long-term clinical outcome.

It was thought that a presence of anti-PLA2R1 or anti-THSD7A was mutually exclusive, however the two serotypes coexist in 1% of MN.[26] The remaining 10-20% of iMN patients who are anti-PLA2R1 and anti-THSD7A seronegative has raised the

hypothesis of other antigens and autoantibodies remained elusive. Cytoplasmatic antigens (e.g. alpha enolase, aldose reductase and superoxide dismutase) are proposed possibilities, but are not widely confirmed.[1,4] In a recent study, *Sethi et. Al* it was identified a third antigen named Neural epidermal growth factor-like1 protein (NELL-1), to be considered as another entity in the serologically defined MN. Their findings suggest that NELL-1 is the second most common antigen (5-10%) in iMN, but more data are needed.[9] Nonetheless, there is still a chance of misclassification of PLA2R1/THSD7A iMN or secondary MN for the rest of the cases.[5] However, anti-PLA2R1 is not commonly present in MN associated to malignancy. Conversely, cancer may be more frequent among patients with anti-THSD7A, but the data are still insufficient to counsel direct malignancy screening approaches in iMN.[4,29,30]

Anti-NELL-1 was recently recognized as another autoantibody in the pathogenesis of iMN.[9] Encoding a 90-kDa protein of 810 amino acids, NELL-1 is strongly expressed in osteoblasts and overexpressed in patients with craniosynostosis.[31,32] It's location in the kidney is higher in the tubules and rare in the glomeruli. However, research in human embryonic kidney cells showed the presence of NELL-1 in the extracellular component and possible deposition in the glomerular basement membrane.[31] In the article by *Sethi et al.*, NELL-1 stained positive and uniformly along the glomerular basement membrane and subepithelium, suggesting that NELL-1 is shed from podocytes rather than being entrapped from circulating antigens or immune complexes.[9] It is seen in older patients and has no gender predisposition. It presents with nephrotic syndrome and in absence of secondary features such as malignancies, autoimmune disease and infections. Further research is needed to determine NELL-1 ultrastructural localization at the podocyte, the potential role of anti-NELL-1 in podocyte adhesion, and its role in disease outcomes.[9] Routinely testing the serum of patients with NELL-1 positive biopsy may enable that, as PLA2R1, it can be used as serological marker.[33]

Serological Detection Methods

Western blotting using protein extracts of human kidneys or extracts of cells transfected with recombinant human cDNA for PLA2R1 was initially used to define the nature of PLA2R1 and THSD7A autoantigens since it's highly sensitive. However, it's unsuitable for clinical use.[7]

Enzyme-linked immunosorbent assays offers the advantage of a simple and prompt quantification, that can be used for a large number of samples.[1] The first commercial method available for diagnosis was CBA-IFA, a cell-based assay using indirect immunofluorescence. After that, ELISA assay (EUROIMMUN Lübeck, Germany) was developed and is currently used routinely in many clinical laboratories to measure total anti-PLA2R1 IgG. The most recent diagnostic tool is a laser bead immunoassay (ALBIA; Mitogen Advanced Diagnosis Laboratory, Calgary, Canada), providing a sensitive and quantitative assay of PLAR2R1 antibodies. This assay was designed to simultaneously measure multiple nephrotoxic antibodies and/or other immunological markers in a single sample, and so probe for the presence of other conditions (SLE, ANCQ-vasculitis, anti-GBM disease).[5,34] Regarding anti-PLA2R1 follow-up, ELISA test is the most suitable test, while CBA-IFA is a more sensitive technique for the detection of very low anti-PLA2R1 levels or when ELISA is inconclusive.[5,35] The manufacturer's definition for anti-PLA2R1 positivity of the commercial ELISA assay is >14 RU/ml.[5] Currently, there is no commercial standard preparation for anti-THSD7A testing.[1,5,27]

Alternatively, kidney biopsy evaluation by immunofluorescence or immunoperoxidase methods on pronase-digested sections of parafine and polyclonal anti-PLA2R1/THSD7A can identify the antigens in the immune deposits, corroborating the diagnosis of PLA2R1/THSD7A-associated iMN.[5,36] Being very sensitive and specific technique to diagnose PLA2R1/THSD7A-associated iMN, it correlates well with serologic tests.[25,36] However, a renal biopsy is a snap-shot in the course of a disease

and may not completely mirror the ongoing immunologic processes in the serum, particularly if these are shifting during the disease progression.[37]

The evidence of anti-PLA2R1 in nephrotic syndrome can be considered as a biomarker for the diagnosis of iMN according to a recent meta-analysis, with a 78% sensitivity (95% CI: 66% to 87%) and a 99% specificity (95% CI: 96% to 100%).[2,38] Noteworthy, the specificity of anti-PLA2R1 antibodies for iMN has been proposed to be around 100% since there is no detections of these antibodies in healthy patients and in non-MN glomerular diseases.[2,7] However, there are very rare cases of secondary MN (sarcoidosis, drugs, lupus nephritis, HBV) which can have positive anti-PLA2R1, but with a much lower prevalence than was mentioned.[2]

This changes the paradigm of kidney biopsy indication, leading to the suggestion that kidney biopsy in positive PLA2R1 patients could be avoided, mainly when there are relative contraindications such as in patients with a single kidney or those with increased risk of bleeding.[2,5,39] Besides aiding diagnosis, it is thought to be of useful for monitoring response in immunosuppressive therapy (IST).[1]

J Floege et. al, in 2019 KDIGO conference report, proposed that kidney biopsy may not be needed in anti-PLA2R1 positive patients with low risk of disease progression and/or a high risk of biopsy-related morbidity.[24,38] Kidney biopsy is indicated in cases of nephrotic syndrome and impaired renal function, once it may identify a crescentic form of MN or a superimposed disease, providing the degree of chronic damage as well.[24]

Moreover, kidney biopsy is crucial for the diagnosis of negative serum anti-PLA2R1/THSD7A iMN, whose secondary causes have been excluded. The glomerular PLA2R1 or THSD7A staining will be decisive for the diagnosis of antigen-associated iMN.[24]

Presenting Features and Risk-Stratification

The discovery of anti-PLA2R1, anti-THSD7A, and recently the anti-NELL1 created a huge expectation on medical practice due to the variability of the clinical outcome, ranging from spontaneous remission of proteinuria to end stage renal disease (ESRD).[39,40]

Since 25-30% of the iMN patients may only present a low-level of proteinuria, usually asymptomatic, these patients have excellent long-term renal survival and do not need IST. In fact, 40% of these patients will not progress and will have a high rate of spontaneous remission, highlighting the indication for conservative management. In the other 60% patients, it is expected to present nephrotic proteinuria in a 2-year time window. This accelerates the progression by 4-fold to an equivalent rate of the initial nephrotic proteinuria range patients.[1] In this circumstance, measuring PLA2R1 autoantigen-autoantibody system is a major step forward in the clinical management of these patients.[40]

On the other end of the disease spectrum, patients with persistent high-grade proteinuria have unequivocally high risk for chronic kidney disease (CKD) including ESRD - 50-75% within 10 years.[1,41] This extensive lag time between proteinuria changes and formal criteria of progression, requires for early sensitive and specific biomarkers to discriminate which patients will progress to CKD. This is of crucial importance because, by delaying treatment, the efficacy is reduced in a disease prone to high posttreatment relapse rate.[1,42] The presence and/or the change in the quantification of circulating levels of PLA2R1 or THSD7A autoantibody may play a promising role in this scenario. This is based on the hypothesis that an immunologic state precedes the proteinuria, (by weeks or months) and the kidney phenotype injury.[1,39,43] An increase in titers of PLA2R1 antibodies has been reported as adverse risk factor for progression of renal failure, especially in older patents. Moreover, this risk was 2.8 times higher in men than in women. Thus, all adult patients with idiopathic

nephrotic syndrome should be screened early on for anti-PLA2R1/THSD7A antibodies together with common causes of secondary MN.[4]

Treatment approaches

The treatment approach focuses proteinuria remission and CKD prevention. Treatment goals also include quality of life measures (for which validated instruments are yet to be developed) as well as prevention of cardiovascular, thromboembolic and infections events, and mortality.[24]

According to KDIGO guidelines, the predictive factors for poor prognosis of iMN are a decrease in glomerular filtration rate at the time of diagnosis, persistent nephrotic proteinuria at 6 months of optimal nephroprotection, male gender, age over 50 years, uncontrolled arterial hypertension, and the presence of interstitial fibrosis and tubular atrophy on renal biopsy.[15,24,44]

Patients with non-nephrotic proteinuria, without an increase in serum creatinine or uncontrolled arterial hypertension, are in general managed by optimal supportive care alone. Dietary sodium restriction and diuretics are essential for edema and blood pressure control (targeted to 125/75 mmHg), and statins for hyperlipidemia. All patients should take angiotensin-converting enzyme inhibitor/angiotensin receptor blocker therapy to minimize proteinuria and to increase the chances of spontaneous remission. Prophylactic anticoagulant therapy (oral warfarin) should be considered if serum albumin is lower than 2.5 g/dL.[1,4,5,15,45]

The criteria for initiating IST are either impairment of kidney function (>50% increase in serum creatinine (□30%) or a level >1.5 mg/dl) or proteinuria refractory to 6 months of supportive care, as defined by the Toronto risk score.[4,15] All patients should undergo screening for infections, and malignancy, accordingly to age and gender, prior to the start of IST.[24]

The highest evidence about remission rates and progression to kidney failure comes from studies using alkylating agents alternating with corticosteroids (Ponticelli regimen) with 10 years of follow-up.[24,45] Based on this evidence, the 2012 Kidney Disease Improving Global Outcomes (KDIGO) guidelines rate this treatment regimen as the first line.[15] As an alternative, KDIGO recommends the use of calcineurin inhibitors (CNi), which in addition to their immunosuppressive actions, also have complementary effects on proteinuria, albeit a higher rate of relapse.[24,46]

Due to the role of B cells in autoantibody production in iMN combined with adverse effects of alkylating agents, corticosteroids, and CNi, increasing observational data have highlighted the effectiveness of rituximab.[47] A lack of randomized, controlled trials excluded for some time the consideration of rituximab as an effective agent.

Van de Logt et. al clearly suggested that anti-PLA2R1 levels may have an important role in the IST decision. The study compared immunological remission rates in patients treated with rituximab or cyclophosphamide. There were no differences between the low or moderate antibody levels. However, in patients with higher titers, cyclophosphamide was more effective in inducing remission than rituximab. Therefore, auto-antibodies titers may be of great value, in particularly to select IST.[48]

An ongoing study (the RI-CYCLO; NCT03018535) aims to address the efficacy of rituximab to the modified Ponticelli regimen. Though, only 76 patients have been recruited, and therefore, results may be limited.

In the GEMRITUX study, rituximab was more effective than placebo inducing remission of proteinuria after 17 months, with a decrease in the levels of circulating anti-PLA2R1 antibody seen at 6 months, and a nonresponse rate to rituximab of 35%.[24,49] Recently, MENTOR study showed that rituximab was superior to cyclosporine in achieving proteinuria remission, and in safety.[50] Adding rituximab to antiproteinuric standard therapy induced a higher remission rate of proteinuria after 6 months of

randomization (35.1% vs. 21%). Because of poor performance of cyclosporin, the MENTOR study suggests that rituximab should be the recommended alternative agent for the treatment of membranous nephropathy when alkylating agents are not suitable (*e.g.*, in women of child-bearing age, those with increased cancer risk due to tobacco exposure, and prior cyclophosphamide).[50] It seems promising, that the MENTOR study will have implications for future guidelines and patient access to this drug.

A potential role as an adjunctive agent with other immunosuppressants remains viable; the STARMEN trial (NCT01955187) that compares the modified Ponticelli regimen with a combination of tacrolimus with rituximab will provide important additional data.

The optimal dose and frequency of administration of rituximab is not yet established.

The dosing of rituximab in the MENTOR study is comparable to the protocol used in the NICE cohort, and it is significantly higher than the cumulative dose received by patients from the GEMRITUX cohort (two 375-mg/m_2 infusions 1 week apart).[49,50] A comparison of these last two cohorts showed that the higher-dose protocol achieved more immunologic and clinical remissions.[51] Rituximab loss into nephrotic urine with insufficient initial dosing may result in subtherapeutic drug levels, and indeed, residual plasma rituximab levels at 3 months were lower in the GEMRITUX cohort compared with the NICE cohort.[51] The field awaits further studies and recommendations guiding the dosing of rituximab in terms of level of nephrosis, autoantibody titer, and dynamic changes in B cells.

Moreover, current treatment options still have a significant unresponsive cohort and a high relapse rate posttreatment which led researchers to consider iMN as a chronic disease that requires continuous therapy.[1,52]

KDIGO guidelines do not consider rituximab as an option for iMN patients, highlighting the need of further clinical trials.[15,45]

Disease monitoring

Data from previous studies shows that antibodies titers start to decrease months before any improvement in proteinuria, immunologic remission precedes clinical remission. For instance, in most anti-PLA2R1/THSD7A positive patients, circulating antibody disappears after 4-6 months of IST.[1,15,45]

The disappearance of circulating anti-PLA2R1 preceded the increase of serum albumin and the reduction of proteinuria in 82% of patients in the MENTOR study, which empowers anti-PLA2R1 as a helpful immunologic biomarker for assessment of disease activity and treatment efficacy.[53]

Once proteinuria is a weak clinical biomarker, checking for antibody levels might aid to anticipate a spontaneous remission, avoiding IST initiation or treatment extension in those with residual proteinuria in whom circulating antibodies have disappeared.[4,25]

Serum anti-PLA2R1 antibody profiles reliably predict response to therapy, and persistent levels at the end of therapy may predict long-term clinical outcome.[5] Complete remission of proteinuria in iMN is associated with good prognosis and long-term kidney survival providing a clear association of the duration of remission and the improved kidney survival.[1,54]

Hoxta et. al presented by multivariate analysis that higher baseline anti-PLA2R1 patients were more likely to progress to nephrotic syndrome and CKD, thereby encouraging prompt initiation of IST.[40] Higher antibody levels have also been described as marker of unlikely to response to therapy. However, it is unknown if the autoantibodies measured at the beginning represent a truly unresponsive patient or a

slower responder.[1] Conversely, low baseline and decreasing anti-PLA2R1 antibody levels strong predict spontaneous remission, thus favoring conservative therapy.[5]

Retrospective studies also suggest that clinical relapse is usually associated with the reappearance of anti-PLA2R1 antibodies.[25,55] Measuring anti-PLA2R1 in patients with recurrence or worsening of proteinuria may help to differentiate between a relapse or other causes of proteinuria.[24]

Indeed, patients with elevated circulating levels of anti-PLA2R1/THSD7A have active immunologic disease and should be considered for immediate IST without waiting 6 months on supportive care alone. While much remains to be known about the correlation between antibodies titers and clinical outcomes, the patients with anti-PLA2R1 in the highest tertile have only a 4% chance of spontaneous immunologic remission if they stay only with supportive care.[4,40,45,55]

Both supportive care and IST require antibody titers monitoring (using quantitative ELISA assay or ALBIA assay) every 1-2 months, until anti-PLA2R1/THSD7A levels become undetectable.[1,15,45]

However, remains to be determined the titer threshold that requires alterations in therapeutic strategy, and the lag time between immunologic and clinical remission.[1]

Persistent levels of anti-PA2R1 before kidney transplant are associated with a high recurrence of iMN in the allograft. There is no clear evidence whether the transplantation should be postponed until the antibodies are negative, and for how long.[24]

Discussion

A decade has been since the discovery of the first antigen PLA2R1, which paved the way to much progress in iMN diagnosis, prognosis and treatment.

In fact, autoantibodies, especially anti-PLA2R1 along with nephrotic syndrome can be considered a biomarker of diagnosis of iMN. Regarding the follow up,

autoantibodies titers have an important role, as a strong predictor of the disease's progression, leaving proteinuria range-based decisions to be abandoned as it seems to be a weak biomarker of iMN for diagnosis and treatment.

The epitope spreading phenomenon also emerges as new promising prognostic biomarkers for clinical outcomes.

Floege et.al, in a KDIGO Controversies Conference report on management and treatment of glomerular diseases, concluded that almost all the KDIGO 2012 membranous nephropathy should be revised based on the recent developments made with podocyte antigen targets of circulating antibodies and on recent clinical studies and trials.[15,24]

Currently, measuring anti-PLA2R1 antibody supports the diagnosis of iMN, forecasts who might evolve to spontaneous remission, aids to monitor disease progression and response to therapy, detects those at risk of progression, and, notably, might help clinicians decide when to reduce/stop treatment.[25]

Upcoming research may explore whether a high positive predictive value of anti-PLA2R1 positivity will obviate the need for a kidney biopsy, help to adapt the therapy strategy and duration for each patient, and its value in assisting for the selection of immunologically active patients for therapeutic studies.[25] Furthermore, as we move towards an autoantibody-specific-disease, the term iMN becomes less appropriate. Instead, "PLA2R1 related MN" or "THSD7A related MN" would be more specific. The term iMN should be restricted to the small percentage of the cases without specific autoantibody and without an identifiable cause for secondary MN.[2]

The levels of circulating antibodies need to be regularly monitored during the follow up to evaluate the long-term outcome disease, efficacy of therapeutic interventions and the risk of disease relapse. Adjustments of interventions according to the evolution of antibodies titers is likely to have a central role in individualizing care of patients with iMN in the future, however the optimal frequency to do this monitoring is not yet defined.

Specific biologic therapy, targeting B cells, as Rituximab, is considered effective and safe in iMN as MENTOR study proved with conclusive evidence that rituximab has a higher efficacy in inducing remission and it's safer than cyclosporine A, one of the recommendations of KDIGO 2012. These results should be a turning point and the future guidelines may likely recommend rituximab over calcineurin inhibitors.[50]

For a promising strategy towards antibody-guided therapy, it is mandatory a standard preparation with all epitopes represented, given that there is now a recognized dominant epitope in the ricin domain and minor epitopes in CTLD1 and CTLD7.[1]

Two promising studies are expected regarding rituximab therapy. The RI-CYCLO trial, which is testing the efficacy and safety of rituximab versus cyclophosphamide/steroids. The STARMEN study randomized tacrolimus for 6-9 months plus rituximab or cyclophosphamide plus steroids. Results are expected to be made public in 2020.[56]

Mapping both anti-PLA2R1 and anti-THSD7A against the clinical phenotypes is crucial in prospective studies and this should help determine whether they can explain more of the clinical diversity of treatment response and outcome.[1]

Considering the creation of a risk stratification or a disease monitoring guide, based on the antibodies assays, becomes imperative to calibrate and adjust the different tests.

The value of immunoadsorption in patients with high titers of antibodies has been questioned. There is currently a small pilot study of anti-PLA2R1 immunoadsorption being undertaken to establish the feasibility of making patients seronegative without IST.[57]

There are still remaining questions: correlation between autoantibody and disease phenotype, explanation for kidney only involvement since the antigen is present in other organs.[1,4]

Conflicts of Interest: The authors declare no conflicts of interest.

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Appendix I – Table I

PRIMARY CAUSES	PLA2R1	70-80%
	NELL-1	5-10%
	THSD7A	2-3%
	Other	5-10%
SECONDARY CAUSES	Infections, Malignancy, autoimmune diseases, drugs, alloimmune diseases.	25-20%

Table 1: Causes of Membranous Nephropathy.

These causes are divided in primary causes (including PLA2R1, THSD7A, NELL-1, others) and secondary.

Appendix II – Journal of Internal Medicine: Instructions to authors

Journal of Internal Medicine (JIM) is an international peer-reviewed journal in continuous publication since 1863. JIM publishes original clinical work within the broad field of general and internal medicine and its sub-specialties and features Original Articles, Reviews and Rapid Communications.

The length of an Original Article should not exceed 5000 words, excluding the reference list. Review articles are usually app. 7000 words.

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JIM publishes Original Articles on clinical and experimental research within the broad fields of general and internal medicine. Original Articles should contain structured abstracts and should not exceed a maximum length of 5000 words of text (not including the abstract, tables, figures, references and online-only material).

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JIM also welcomes Review Articles at the forefront of medical research. These Articles should include an informative abstract where relevant key findings are presented. Please remember that a well-written and informative abstract is a teaser for further reading of the full article. Furthermore, we recommend you to include one or two tables summarizing important results as well as illustrative figures. The recommended length is approximately 7000 words (not including the abstract, tables, figures, references and online-only material).

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Rapid Communications are concise papers (not exceeding 1500 words) representing novel and completed work that is of unusually high priority and significance. Rapid Communications should include a structured abstract.

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JIM publishes Educational Updates on Current Issues (for example, “Lessons in biostatistics”). Educational Updates on Current Issues should not include an abstract and should be a maximum length of 5000 words (not including tables, figures, references and online-only material).

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Submission of manuscripts

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Authors for whom English is a second language may choose to have their manuscript professionally edited before submission, in order to (i) improve grammar, spelling and punctuation, (ii) improve clarity and resolve any ambiguity and (iii) improve word choice and ensure that the tone of the language is appropriate for an academic journal. Of note, all accepted articles are edited for language by our professional language editor at no cost to the author.

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Manuscripts should be submitted online at ScholarOne Manuscripts. Full instructions and support are available on the website, and a user ID and password can be obtained on the first visit. All parts of the manuscript must be available in an electronic format; Microsoft Word or generic RTF are recommended for text, and JPEG, GIF, TIFF, EPS, PNG, Microsoft PowerPoint or Excel for graphics (see section Tables and figures). For a manuscript to be considered for

publication, the author should suggest at least four reviewers. It is recommended that, where possible, figures and tables should be placed within a single word file. Apple Macintosh users should ensure compatibility by submitting files with correct PC filename suffixes (e.g. '.doc' for Microsoft Word).

All material should conform to Uniform Requirements for Manuscripts Submitted to Biomedical Journals. The latest version of the rules of the ICMJE (The Vancouver Group) may be found in Medical Education 1999; 33: 66–78. Manuscripts should preferably be written in British English, although American English is also acceptable. Abbreviations should be kept to a minimum and an abbreviation list included, if necessary.

The SI system must be used for all units (for guidance, see physics.nist.gov).

- *Cover letter*

When submitting a manuscript a cover letter must be included, providing contact information for the corresponding author and stating whether the authors have published or submitted any related papers from the same study.

- *Arrangement of the manuscript*

All pages (including references, tables and their captions, figure captions and, where possible, figures) should be saved in a single electronic file.

The manuscript should include the following: (i) Title page, (ii) Abstract where applicable, (iii) Keywords (iv) Main text (Introduction, Materials and methods, Results, Discussion and Conclusion), (v) Conflict of interest statement, (vi) Acknowledgments, (vii) References, (viii) Figure legends, (ix) Tables and their captions and (ix) Figures.

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Title. To improve searchability, the title should be informative, rather than descriptive, and clearly state the key message of the paper. A full sentence is preferred.

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Correspondence. The last page of the reference list should include the name and address, including email address, of the author to whom correspondence, including requests for offprints, should be sent. The corresponding author is advised to select an alternative proof reader from among the co-authors; the name and email address of this 'second' author should be provided.

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