Anti-PLA2R: a non-invasive serological biomarker in lieu of biopsy

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Overview

Accurate diagnosis of the autoimmune kidney disease primary membranous nephropathy (pMN) is crucial for therapy decision-making. Autoantibodies against phospholipase A2 receptors (PLA2R) play a central role in the pMN diagnostic algorithm in the updated KDIGO Clinical Practice Guideline for the Management of Glomerular Diseases. Diagnosis of pMN can now be made without biopsy if anti-PLA2R antibodies are present alongside the clinical symptoms. Thus, in many cases, patients can be spared expensive, invasive and stressful biopsy procedures. Anti-PLA2R is also a key parameter for therapy monitoring and disease course prognosis.

Membranous nephropathy

Membranous nephropathy (MN) is chronic inflammatory disease of the kidney corpuscles (glomeruli), which can potentially lead to kidney failure. It is characterised by thickening of the glomerular capillary walls due the deposition of immune complexes. The deposits damage the podocytes and impair the permeability of the glomerular basement membrane, leading to proteinuria. If protein excretion in the urine is excessively high, nephrotic syndrome with hypoproteinaemia (reduced blood protein concentration), hyperlipidaemia (increased blood lipid levels) and oedema can develop.

The primary form, pMN, has an autoimmune genesis and is the leading cause of nephrotic syndrome in adults. It occurs more often in middle-aged and elderly people and hardly in children. It has an insidious onset and a variable disease course. About one third of patients experience spontaneous remission, one-third remain proteinuric with stable renal function, and one third progress to end-stage kidney disease.

Secondary membranous nephropathy (sMN) develops as a result of an underlying condition such as an infection, another autoimmune disease, a tumour or drug poisoning. The secondary form accounts for 20-30 % of MN cases. Diagnostic differentiation of pMN and sMN is vital due to differing treatment regimens. Patients with pMN are mainly treated with immunosuppressives, while in sMN the treatment focuses on the underlying disease. Correct and early diagnosis can prevent unnecessary diagnostic procedures or drug treatments.

Autoantibodies in pMN

pMN is characterised by autoantibodies against the podocyte proteins PLA2R and thrombospondin type-1 domain-containing 7A (THSD7A). These

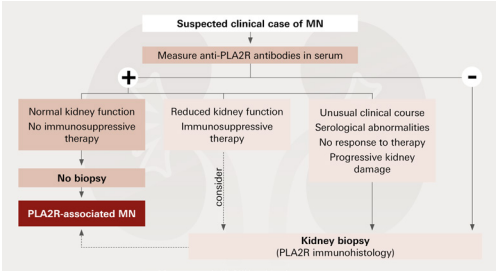

Figure 1. Diagnostic guideline for suspected pMN

autoantibodies were first identified as highly specific serological markers for pMN in 2009 and 2014, respectively. Even though the exact pathogenic mechanisms for the development of pMN have not yet been conclusively clarified, it is now widely accepted that the autoantibodies play a pathogenic role. Anti-PLA2R antibodies occur in pMN with a prevalence of 70 % to 80 %, while anti-THSD7A antibodies have a prevalence of up to 10 %. Anti-THSD7A antibodies are found predominantly in anti-PLA2R-negative pMN patients, and thus play a complementary role in pMN serodiagnostics. The specificity of both autoantibodies for pMN is extremely high.

Diagnostic algorithm

pMN should always be considered in the diagnostic workup in cases of unexplained proteinuria and nephrotic syndrome. The new KDIGO guideline recommends a differentiated approach for pMN diagnostics (Figure 1). In suspected clinical cases, anti-PLA2R is measured in serum. A positive result enables a diagnosis to be established without biopsy in patients with nephrotic syndrome and normal kidney function. A biopsy should be considered for patients with reduced kidney function who are already receiving immunosuppressive therapy, as the antibody titer may be reduced by the treatment. Biopsy may also be required in patients with an unusual clinical course, a rapid decrease in glomerular filtration rate (eGFR), abnormal serological results for other markers such as anti-nuclear antibodies, therapy unresponsiveness and progressive kidney injury, or persistent nephrotic syndrome with disappearance of anti-PLA2R. With a negative anti-PLA2R result and continued suspicion of pMN, a biopsy is also recommended. In the early phase of the disease, the antibodies can be bound in the kidney and are therefore not detectable in the serum. Then, the diagnosis of PLA2R-associated pMN can be confirmed by kidney biopsy with immunohistological detection of glomerular PLA2R immune complex deposits.

Disease activity monitoring

In anti-PLA2R-positive patients, the KDIGO guideline recommends follow-up measurements of the anti-PLA2R antibody level every three to six months to aid patient management, with a shorter interval advised for patients with high antibody titers.

The anti-PLA2R antibody level reflects the clinical activity of pMN. Patients with acute nephrotic syndrome (high proteinuria) show high anti-PLA2R titers. With spontaneous or treatment-induced remission (low proteinuria), the titer decreases to below the detection limit. A relapse of pMN is associated with an increase in the antibody level.

The decrease or increase in the anti-PLA2R antibody level usually precedes the clinical change by months (Figure 2). Thus, phases of clinical remission and relapses can be predicted by regular monitoring of the antibody level. A high anti-PLA2R titer is among the risk factors for progression to renal failure in pMN patients.

Therapy decision-making

Treatment regimens for pMN include calcineurin inhibitor plus glucocorticoids, rituximab, or cyclophosphamide plus glucocorticoids, depending on the patient's risk profile. The anti-PLA2R antibody level provides guidance on the necessity and the type of immunosuppressive therapy and allows assessment of a patient's response to the therapy. Patients who successfully respond to treatment show a strong reduction in the antibody titer months before the reduction in the clinical parameter proteinuria. The change is often detectable after just three months, enabling the therapy to be adjusted accordingly. A high antibody titer is associated with a lower likelihood of remission via treatment, which can be taken into account in the therapy choice.

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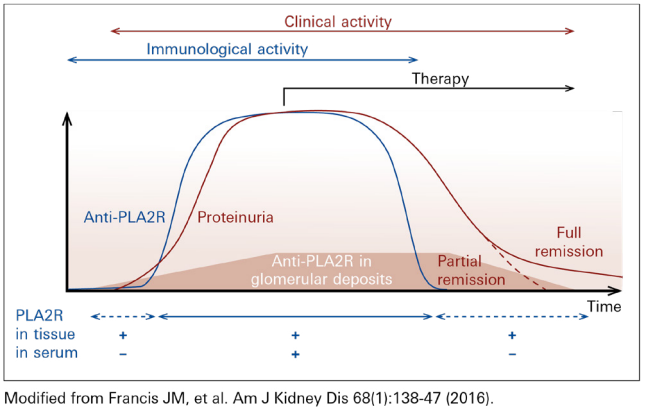

Modified from Francis JM, et al. Am J Kidney Dis 68(1):138-47 (2016).

Figure 2. Representation of immunological (anti-PLA2R) and clinical (proteinuria) activity in pMN

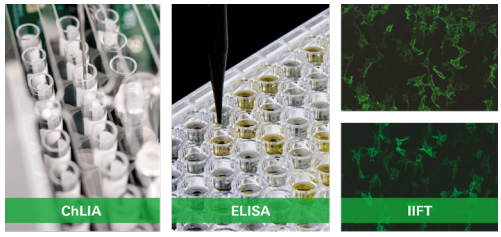

Figure 3. Different test methods for detection of anti-PLA2R antibodies

Risk assessment after transplantation

In pMN patients who undergo kidney transplantation, the disease recurs in around 40 % of cases after the organ transplant. The risk of recurrence is especially high if anti-PLA2R antibodies persist over a time period of six months following the transplant. The KDIGO recommends measuring anti-PLA2R both pre-transplantation for risk assessment and post-transplantation for monitoring the disease course and deciding on the requirement for immunosuppressive therapy.

Diagnostic tests

Autoantibodies in pMN can be determined using exclusive test systems from EUROIMMUN (Figure 3). The indirect immunofluorescence test (IIFT) enables qualitative to semiquantitative detection of anti-PLA2R or anti-THSD7A antibodies using transfected cells expressing the corresponding antigen on their surface. A control substrate comprising non-transfected cells serves as an internal control. Determining both antibodies in parallel or via a two-step strategy, in which anti-PLA2R-negative sera are subsequently tested for anti-THSD7A, can increase the serological detection rate for pMN.

Anti-PLA2R antibodies can be measured quantitatively using ELISA or chemiluminescence immunoassay (ChLIA). In the ELISA, purified recombinant receptor is coated onto the wells of a microplate, while the chemiluminescence immunoassay (ChLIA) utilises magnetic particles coated with the antigen. The quantitative antibody measurement with ELISA or ChLIA is highly suited to disease and therapy monitoring and can be performed efficiently at high throughput.

The IIFT, ELISA and ChLIA procedures can be automated using different devices according to the laboratory's requirements, providing increased standardisation and streamlining of the analyses.

Outlook

The incorporation of anti-PLA2R into the KDIGO guideline as a stand-alone serodiagnostic marker for pMN is a huge benefit to both patients and clinicians, as it eliminates the need to perform a biopsy in many cases. The new recommendation underscores the reliability and specificity of anti-PLA2R. Anti-PLA2R is complemented by anti-THS7DA, which can increase the diagnostic detection rate. Although data on anti-THS7DA in disease and therapy monitoring is still lacking, these antibodies may also play a future role in patient management. Furthermore, a plethora of other autoantigenic targets in pMN has been identified by scientific research in recent years. Given the rapid pace of discovery, it is anticipated that additional serological parameters will enter the clinical routine in the coming years, further enhancing the diagnostic and monitoring possibilities for pMN.