Anti-PLA2R antibody detection in primary membranous nephropathy:

now recommended by KDIGO

by Dr Jacqueline Gosink

Autoantibodies against M-type phospholipase A2 receptors (PLA2Rab) are a central biomarker for diagnosis and monitoring of primary membranous nephropathy (pMN). PLA2Rab detection has recently been incorporated into the new KDIGO Clinical Practice Guideline for the Management of Glomerular Diseases. Diagnosis of pMN can now be made without biopsy if PLA2Rab are present alongside corresponding clinical symptoms. PLA2Rab determination is also recommended by the KDIGO for disease course prognosis, therapy monitoring and assessment of pMN kidney transplant patients.

Primary membranous nephropathy

Membranous nephropathy (MN) is a chronic inflammatory disease of the glomeruli which is accompanied by a progressive impairment of kidney function. It is the leading cause of nephrotic syndrome in adults, occurring in all ethnic groups and in both sexes.

MN is divided into the primary form (pMN), which has an autoimmune origin and accounts for approximately 70–80% of cases, and the secondary form (sMN), which develops from an underlying condition such as infection, medication, drug or toxin intake, another autoimmune disease or cancer. Differentiation of the two forms is critical owing to different therapy regimens. Although pMN is treated mainly with immunosuppressives, sMN therapy is targeted at the underlying cause.

pMN has an insidious onset and a variable disease course. Around one-third of patients experience spontaneous remission, one third remain proteinuric with stable renal function, and one third progress to end-stage kidney disease. pMN can also recur following kidney transplantation.

Autoantibodies in pMN

pMN is characterized by autoantibodies against the podocyte proteins M-type phospholipase A2 receptors (PLA2R) and thrombospondin type-1 domain-containing 7A (THSD7A). Anti-PLA2R antibodies (PLA2Rab) occur in pMN with a prevalence of 70–80%, whereas anti-THSD7A antibodies (THSD7Aab) have a prevalence of up to 10%. THSD7Aab are found predominantly in PLA2Rab-negative patients and thus play a complementary role in serodiagnostics. The specificity of both antibodies for pMN is very high.

The autoantibodies play a pathogenic role in pMN by forming immune-complex deposits that 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 hypoproteinemia, hyperlipidemia and edema can develop.

Kidney Disease: Improving Global Outcomes guideline

The first Kidney Disease: Improving Global Outcomes (KDIGO) guideline on glomerular diseases was published in 2012 to aid management of primary and secondary glomerular diseases, which are among the most common causes of irreversible kidney damage. In the intervening years, pMN has been unequivocally demonstrated to be an autoimmune disease. The new 2021 guideline incorporates updated strategies for monitoring pathogenic autoantibodies in the diagnosis, prognosis and treatment of pMN.

Diagnosis

In cases of unexplained proteinuria and nephrotic syndrome, pMN should always be taken into account in the diagnostic workup. Kidney biopsy is usually considered the gold standard for evaluation of glomerular diseases. However, it can now be omitted for the diagnosis of pMN in patients with corresponding

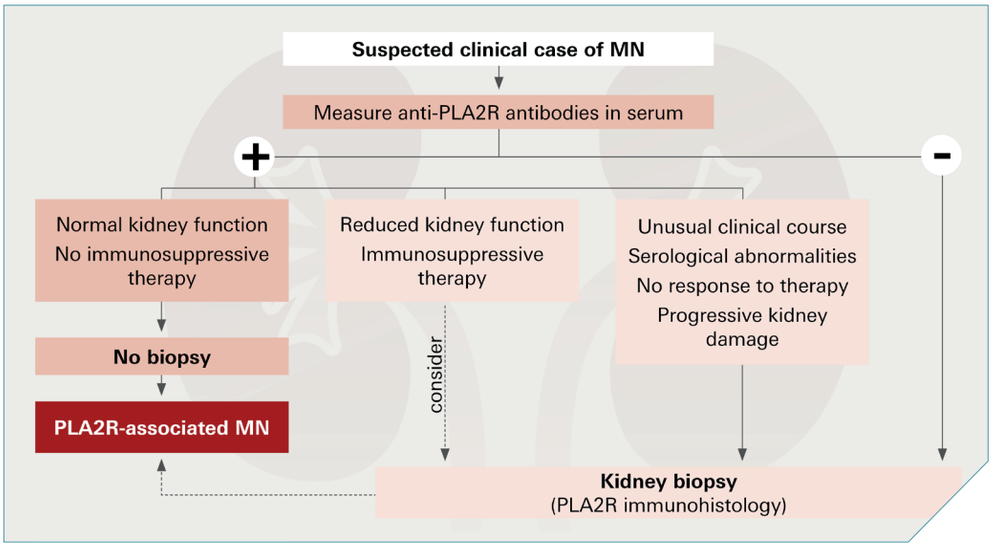

Figure 1. Diagnostic guideline for suspected primary membranous nephropathy
(EUROIMMUN)
MN, membranous nephropathy; PLA2R, phospholipase A2 receptors

clinical features and a positive PLA2Rab test (Fig. 1). Thus, patients can avoid the stressful invasive procedure and its associated risks such as pain and bleeding.

A biopsy should still be considered for patients with reduced kidney function who are receiving immunosuppressive therapy, as the antibody titre 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 despite disappearance of PLA2Rab.

With a negative PLA2Rab result and continued suspicion of pMN, a biopsy is also recommended. In the early phase of the disease, 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 immunohistochemical detection of glomerular PLA2R immune-complex deposits.

Prognosis

In patients with PLA2R-associated pMN, clinical and laboratory criteria are used to assess the risk of progressive loss of kidney function. According to the KDIGO guideline, PLA2Rab should be measured at 3- to 6-month intervals, with the shorter interval advised for patients with a high PLA2Rab titre at baseline. The PLA2Rab titre reflects the clinical activity of pMN, whereby an increase or decrease in the antibody level usually precedes the clinical change by months (Fig. 2). Disappearance of PLA2ab is mostly followed by clinical remission. Continued presence of PLA2Rab indicates persistent disease activity. High initial titres are associated with a lower likelihood of spontaneous remission and a higher likelihood of a non-response to low-dose therapy.

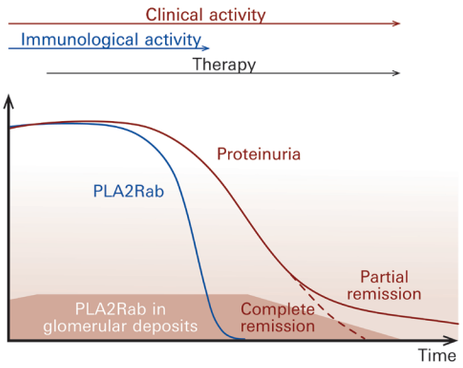

Figure 2. Representation of immunological (anti-PLA2R antibodies) and clinical (proteinuria) activity in pMN
(EUROIMMUN)

Treatment

Treatment for pMN follows a risk-based strategy. Owing to potential adverse effects, immunosuppressive treatment is restricted to patients at risk of progressive kidney injury. Medications include rituximab, calcineurin inhibitor, cyclophosphamide, glucocorticoids and combinations thereof, depending on the patient's risk profile.

PLA2Rab-positive pMN patients who successfully respond to treatment show a strong reduction in the antibody titre several months before the reduction in the clinical parameter proteinuria. Therefore, monitoring of PLA2Rab levels at 6 months after start of therapy is recommended for early evaluation of treatment response in patients and is useful for guiding adjustments to therapy. In most cases the treatment response occurs within 3 months after the start of therapy.

Special situations

Immunological monitoring is particularly valuable in patients with initial relapse after therapy-induced remission. Positive PLA2Rab in a period of clinical remission is evidence for resistant disease. Therefore, in patients with PLA2Rab, the antibody titre should be evaluated at the time of remission and relapse.

Resistant disease can be defined by the persistence of PLA2Rab at high or unchanged levels after the first line of immunosuppressive therapy. In this case, further treatments should be evaluated. Persistent proteinuria is not sufficient to define resistance, as it can persist for 12 to 24 months after start of therapy. Some patients demonstrate partial remission of proteinuria and persistent low-level PLA2Rab. These patients can often refrain from immunosuppressive therapy but should be followed carefully. >>

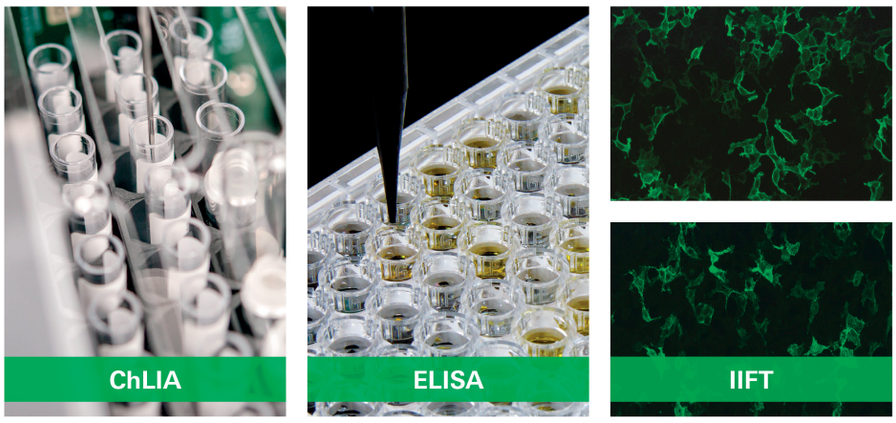

Figure 3. Different test methods for detection of anti-PLA2R antibodies (EUROIMMUN)
ChLIA, chemiluminescence immunoassay; ELISA, enzyme-linked immunosorbent assay; IIFT, indirect immunofluorescence test.

Kidney transplant recipients

In pMN patients who require a kidney transplant, KDIGO recommends measuring PLA2Rab both pre-transplantation for risk assessment and every 1 to 3 months post-transplantation for monitoring the disease course and deciding on the requirement for immunosuppressive therapy. An absence of PLA2Rab at the time of transplantation predicts a low risk of disease recurrence. Persistently high or increasing titres of PLA2Rab following transplantation are associated with a high risk of recurrence.

Diagnostic tests

Autoantibodies in pMN can be determined using test systems available exclusively from EUROIMMUN (Fig. 3). The indirect immunofluorescence test (IIFT) enables qualitative to semiquantitative detection of PLA2Rab or THSD7Aab 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 PLA2Rab-negative sera are subsequently tested for THSD7Aab, can increase the serological detection rate for pMN.

PLA2Rab can be measured quantitatively using ELISA or chemiluminescence immunoassay (ChLIA). In the ELISA, purified recombinant receptor is coated onto the wells of a microplate, whereas the ChLIA uses 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 standardization and streamlining of the analyses.

Future developments

Incorporation of PLA2Rab into the KDIGO guidelines has enriched the diagnosis and monitoring of pMN, reducing the number of costly and arduous biopsies required. Further research will aim to elucidate the role of PLA2Rab as a pre-clinical marker. A deeper understanding of THSD7Aab will clarify the clinical usefulness of this parameter in diagnosis and follow-up. In particular, THSD7Aab may be associated with a higher risk of malignant tumours than PLA2Rab. Patients who are double negative for PLA2Rab and THSD7Aab are likely to harbour other pathogenic antibodies. A variety of potential antigenic targets has been discovered by scientific research in recent years. Thus, additional serological parameters for pMN will probably enter the diagnostic scene in the coming years.

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