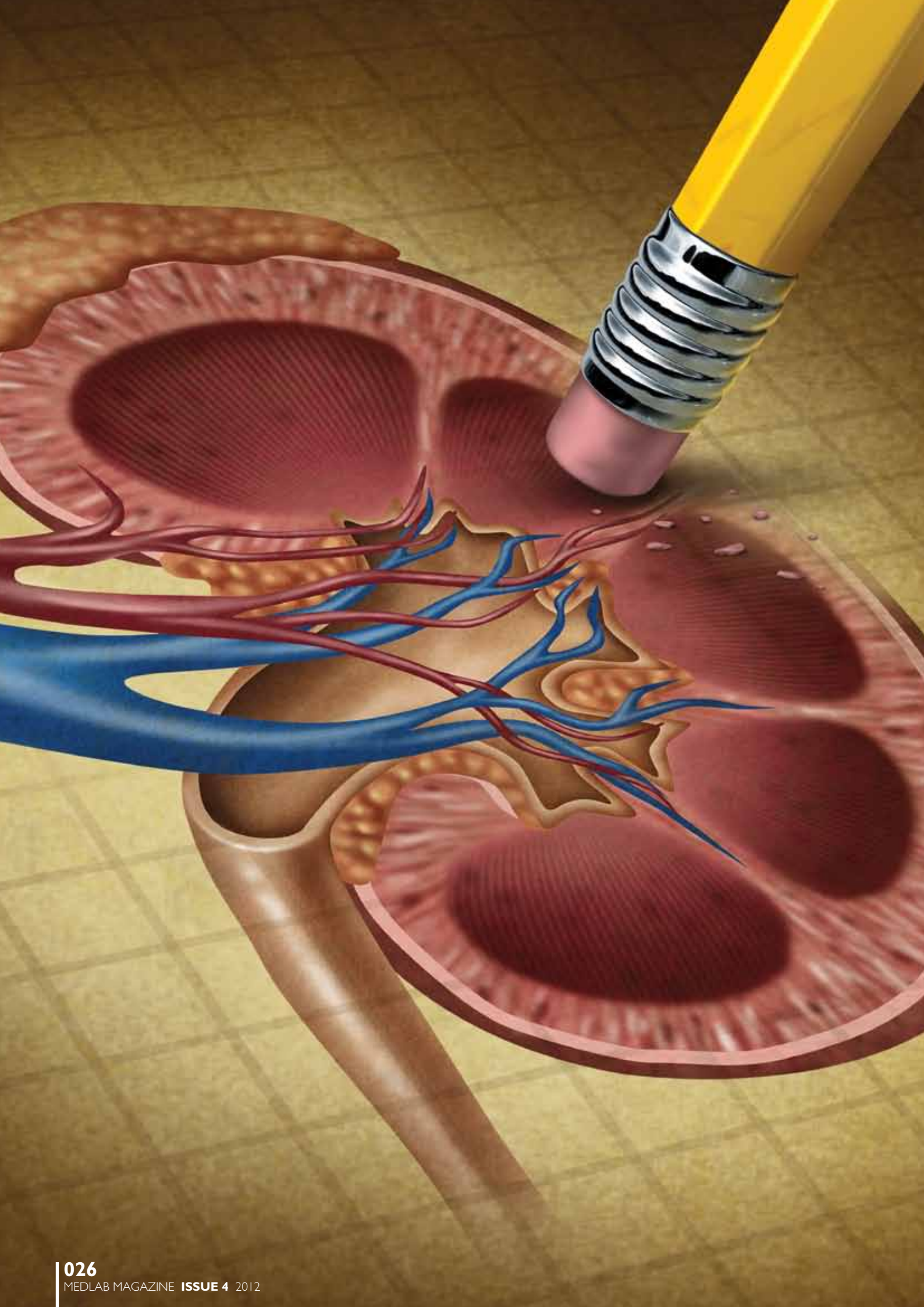

AUTOANTIBODIES AGAINST PHOSPHOLIPASE A_2 RECEPTORS: A NEW NEPHROLOGICAL BIOMARKER

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OVERVIEW

Autoantibodies against phospholipase A_2 receptors (PLA_2R) are a new and highly specific diagnostic marker for primary membranous glomerulonephritis (MGN). They were identified only recently as frequently occurring autoantibody parameters in patients with this debilitating kidney disease. The rapid development of simple serological tests to detect anti-PLA_2R antibodies has enriched the diagnosis and monitoring of primary MGN and advanced understanding into the mechanisms of its pathogenesis.

PRIMARY MEMBRANOUS GLOMERULONEPHRITIS

Primary MGN, also known as idiopathic membranous nephropathy or IMN, is a chronic inflammatory autoimmune disease of the blood-filtering structures of the kidneys (glomeruli). It is accompanied by a progressive reduction in renal function. The disease manifests with the symptom complex nephrotic syndrome, which is characterised by heavy proteinuria, hypoalbuminemia, hyperlipidemia, edema and lipiduria. Primary MGN is one of the leading causes of nephrotic syndrome in Caucasian adults. As proteinuria increases, so does the long-term risk of kidney failure with major morbidity and mortality, especially from thromboembolic and cardiovascular complications. Around a third of patients' progress to end-stage renal disease, a third exhibit persistent proteinuria without progression to renal failure, and the remainder experience spontaneous remission. Primary MGN is prevalent in all ethnic groups and in both genders, with Caucasian men over 40 years old being the most frequently affected.

A CHALLENGE TO DIAGNOSIS

The diagnosis of primary MGN is demanding, as the disease must be differentiated from other nephropathies, especially from secondary MGN, which is triggered by an underlying cause such as a malignant tumour, an infection, drug intoxication or another autoimmune disease such as systemic lupus erythematosus. Of all MGN cases, 20-30% are of secondary genesis, while the remaining 70-80% are classified as primary. Reliable differentiation of the two forms is critical due to different treatment regimes: primary MGN is treated with immunosuppressives, while therapy for secondary MGN is targeted at the underlying disease.

MGN is diagnosed by kidney puncture followed by histological examination or electron microscopy of the tissue to identify the characteristic glomerular immune deposits. To obtain a definite diagnosis of primary MGN, secondary causes must be excluded, which involves additional time-consuming and often invasive procedures, for example tumour screening. Moreover, in some patients MGN appears before the secondary cause is even detectable, adding an extra layer of complexity to diagnosis and therapeutic decision-making. Primary MGN must also be differentiated from other autoimmune diseases with kidney involvement, for example lupus nephritis, vasculitides associated with antibodies against neutrophil cytoplasm (ANCA) and Goodpasture's syndrome. The availability of a reliable serological test to support the diagnosis of primary MGN has been elusive up until now due to lack of knowledge about the target antigen.

ANTI-PLA2R AUTOANTIBODIES – A NOVEL MARKER

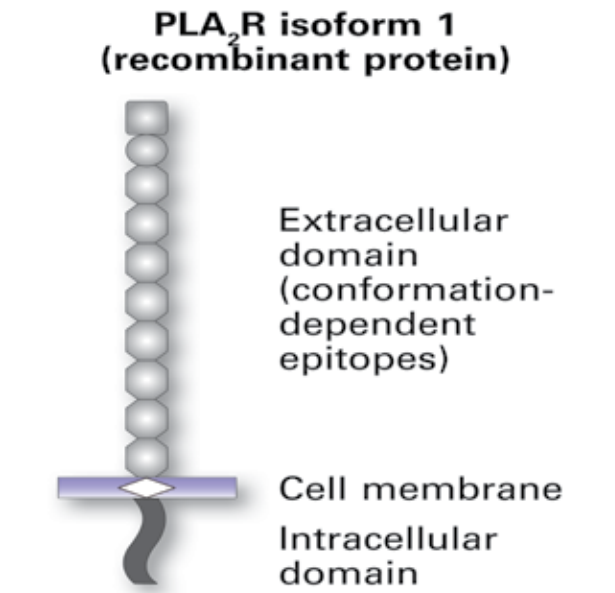
Autoantibodies against PLA_2R were first discovered and described in patients with primary MGN in 2009. PLA_2R are transmembrane glycoproteins (*see figure 1*) which are expressed in human glomeruli on the surface of podocytes and are involved in regulatory processes in the cell following phospholipase binding (*see figure 2*). Currently two main groups of PLA_2R are known, namely type M and N. Type M have been identified as the major target antigen of autoantibodies. In patients with primary MGN, antigen-antibody complexes form deposits in the glomerular basement membrane, where they trigger complement activation with overproduction of collagen IV and laminin. This causes damage to the podocytes via destruction of the cytoskeleton and broadening of the basement membrane. As a result, protein enters the primary urine, giving rise to proteinuria and other symptoms.

Circulating autoantibodies of class IgG against PLA_2R are present in the serum of up to 70-80% of patients with primary MGN, whereas they are not found in healthy blood donors or patients with other kidney diseases such as lupus nephritis or IgA nephritis. The high sensitivity and specificity of anti-PLA_2R for primary MGN make this parameter ideal for use as a diagnostic marker. Furthermore, the antibody concentration shows a strong correlation with the clinical status. Antibody levels are high in the nephrotic phase of primary MGN, decrease or disappear during spontaneous or treatment-induced remission and increase again with relapse. Thus, anti-PLA_2R analysis is also suitable for long-term monitoring of the disease activity and the response to therapy.

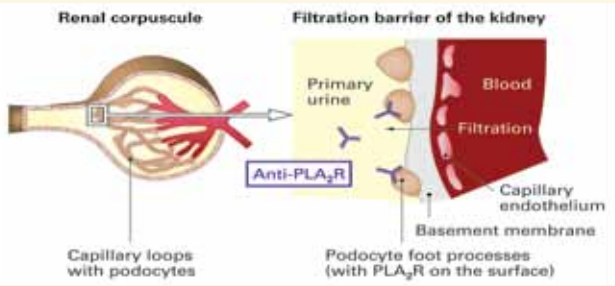
INNOVATIVE ANTI-PLA2R TEST SYSTEMS

Following the identification of PLA_2R as the target antigen of autoantibodies in primary MGN, an indirect immunofluorescence test (IIFT) for their detection was rapidly developed. The IIFT utilises a BIOCHIP of transfected human cells expressing recombinant PLA_2R as the antigenic substrate to provide monospecific antibody detection (*see figure 3*). A second BIOCHIP containing non-transfected cells serves ➔

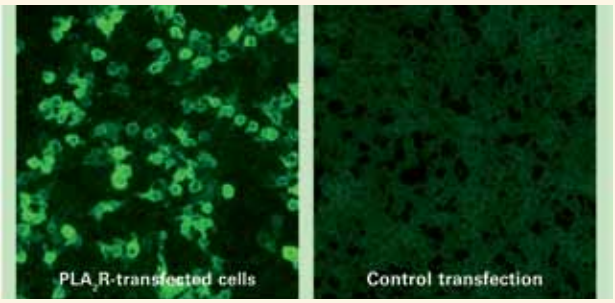
▼**FIGURE 1:** Structure of PLA_2R



▼**FIGURE 2:** Binding of anti-PLA_2R to kidney podocytes

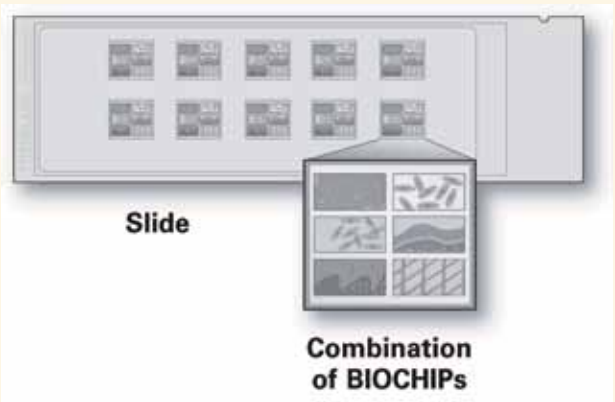


▼**FIGURE 3:** Positive reaction in Anti-PLA_2R IIFT



▼**FIGURE 4:** Anti-PLA_2R ELISA

▼**FIGURE 5:** BIOCHIP Mosaic



as a control. Since its introduction, the recombinant cell-based IIFT has rapidly become established as the gold standard for the serological diagnosis of primary MGN. An ELISA based on purified recombinant PLA_2R has also been developed (*see figure 4*) and shows the same high-quality characteristics as the IIFT. The ELISA is particularly useful for disease monitoring as it offers quantification of antibody levels in patient sera.

Both the IIFT and the ELISA are fast and simple to perform and are suitable for use in any diagnostic laboratory. In the IIFT, further substrates can be included in the BIOCHIP Mosaics (*see figure 5*) to enable the parallel investigation of other autoantibodies that are relevant in nephrology, for example anti-double-stranded DNA and anti-nucleosome antibodies (lupus nephritis), ANCA (renal vasculitis) and anti-GBM antibodies (Goodpasture's syndrome). Extensive automation options are available for both test methods.

HIGH DIAGNOSTIC SENSITIVITY AND SPECIFICITY

The Anti-PLA_2R IIFT has been employed in numerous clinical studies and demonstrates a specificity of 100% and a sensitivity of around 50-80% depending on the cohort. In a retrospective clinical study the IIFT yielded a sensitivity of 52% in a cohort of 100 patients with biopsy-proven primary MGN and a specificity of 100% with respect to healthy controls and patients with secondary MGN or non-membranous glomerular injury. In the first prospective clinical study the sensitivity amounted to 82% in patients with biopsy-proven MGN where no secondary cause could be found. The difference in sensitivities obtained in different study panels may be due to factors such as disease remission and therapy status of the individuals, which can influence the antibody results, especially when studies are performed retrospectively. Further, while PLA_2R has been identified as the major target antigen in primary MGN, it is also possible that a small proportion of patients exhibit antibodies against alternative target antigens. In-depth studies using these test systems are underway to elucidate the role of anti-PLA_2R in primary MGN and to further explore their relationship with clinical disease activity, proteinuria, therapeutic results, etc.

POTENTIAL APPLICATION IN TRANSPLANT PATIENTS

Up to 40% of patients with primary MGN who undergo renal transplantation experience a recurrence of the MGN following transplantation. The anti-PLA_2R levels were tracked in one such patient using the IIFT. Antibodies were detected both before and three months after transplantation. Immunosuppressive therapy with rituximab resulted in a drop in the antibody concentration and also the level of proteinuria. This raises the intriguing possibility of using anti-PLA_2R to screen MGN patients awaiting transplantation to assess if they might benefit from additional immunosuppressive medication. More detailed studies will determine the applicability of this concept.

CONCLUSION

The recent identification of autoantibodies against PLA_2R as biomarkers for primary MGN and the development of innovative assays to detect them represents a landmark in nephrological diagnostics. Analysis of anti-PLA_2R using simple laboratory tests is extremely useful for the diagnosis of primary MGN, the differentiation of primary and secondary forms, the monitoring of disease activity and the evaluation of treatment. As serological screening is non-invasive and less time-consuming than biopsy, both healthcare providers and patients stand to benefit from this milestone development.

REFERENCES

References available on request (magazine@informa.com)