



# AUTOANTIBODY DIAGNOSTICS IN AUTOIMMUNE NEPHROPATHIES

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## INTRODUCTION

Autoimmune dysfunctions are the 'bête noire' in a range of debilitating nephropathies. Autoimmune-mediated damage to the kidneys can be triggered by autoantibodies directed against specific renal structures or proteins resulting in diseases such as primary membranous nephropathy (MN) or Goodpasture's syndrome. Moreover, secondary damage to the kidney can be part of the wide-reaching effects of systemic autoimmune diseases such as vasculitis or systemic lupus erythematosus (SLE), which are characterized by non-organ-specific autoantibodies. A variety of innovative and highly specific and sensitive autoantibody tests that have been developed in the past years are currently available to identify autoimmune kidney diseases at an early stage. The most important ones are described below.

## ANTI-PLA2R AND ANTI-THSD7A ANTIBODIES IN PRIMARY MEMBRANOUS NEPHROPATHY

Membranous nephropathy (MN) is a chronic glomerular disease characterized by in-situ formation of immune complexes in the glomerular basement membranes followed by complement activation. Damage of podocytes results in proteinuria, and frequently in a nephrotic syndrome. Most cases (70-80%) of MN belong to the idiopathic or primary form. Phospholipase A2 receptor (PLA2R) autoantibodies are highly specific for the primary form of MN and are found in the serum of around 75% of patients at baseline. For the determination of PLA2R autoantibodies, two standardized assays are available that are highly suitable for routine diagnostic purposes: a recombinant cell-based indirect immunofluorescence assay (RC-IFA) and an enzyme-linked immunosorbent assay (ELISA). RC-IFA uses the human cell line HEK293 overexpressing full-length human PLA2R as substrate. The PLA2R-positive cells are arranged in a biochip format in combination with control-transfected cells in one incubation field (Fig. 1). Using this assay, antibodies to PLA2R were detected with maximal specificity (100%) and with a sensitivity of 77 % in a cohort of 275 biopsy-proven pMN patients. PLA2R antibody titers decline in the course of spontaneous remission and during successful therapy. For the accurate quantification of autoantibody concentrations an ELISA based on the recombinantly produced extracellular domain of PLA2R has recently been developed. In a large cohort of clinically well-characterized patients, this assay revealed very high sensitivity with respect to RC-IFA (96.5 %) at a set specificity of 99.9 %. In addition to differential diagnosis of MN, the ELISA-based quantification of PLA2R autoantibodies allows assessment of disease activity and severity as well as prediction of disease outcome (remission, relapse) and risk of recurrence of MN after kidney transplantation. Moreover, it facilitates treatment decisions and monitoring of response to treatment.

Very recently, thrombospondin type-1 domain-containing 7A (THSD7A) was discovered as a second antigenic target in approx. 2.5 % to 5 % of patients with idiopathic MN. Importantly, these autoantibodies have exclusively been found in the anti-PLA2R-negative cohort suggesting that these patients represent a distinct disease subgroup. No reactivity against THSD7A was observed among healthy controls and among patients with other proteinuric or renal autoimmune diseases. As PLA2R, THSD7A is an N-glycosylated high molecular mass protein expressed on the podocyte membrane. Similar to PLA2R antibodies, an association between THSD7A antibody levels and disease activity is suggested. Further studies using THSD7A-based RC-IFA are currently ongoing.

## ANTI-GBM ANTIBODIES IN GOODPASTURE'S SYNDROME

Autoantibodies to glomerular basement membranes (GBM) are highly specific and sensitive markers for Goodpasture's syndrome, which is characterized by rapidly progressive glomerulonephritis (GN) and lung hemosiderosis. GBM antibodies have shown to be crucial for an early diagnosis of the disease and are detected in more than 90% of patients presenting with and in over 60% of patients without lung involvement. The relevant antigenic target is the non-collagenous I (NC1) domain of the alpha-3 chain of type IV collagen within the GBM that can easily be detected by indirect immunofluorescence assays (IFA) using cryo-sections of primate kidney or by various monospecific assays based on the purified NC1 domain (e.g. ELISA, line immunoassays, microdots in IFA). Since the clinical progression of the disease correlates with antibody concentration, with high-titers indicating an unfavourable prognosis, ELISA-based quantification of GBM autoantibodies enables a monitoring of the course of disease.

Approximately 20 % to 35 % of patients with anti-GBM antibodies also have anti-neutrophil cytoplasmic autoantibodies (ANCA) mostly with specificity for myeloperoxidase (MPO). Therefore, it is recommended that anti-GBM and ANCA should be analyzed in parallel in patients with renal disease (Fig. 2).

## ANTI-NEUTROPHIL CYTOPLASMIC ANTIBODIES (ANCA) IN RENAL VASCULITIS

ANCA are characteristic for autoimmune vasculitis, which manifests with a range of symptoms in different organs, including the kidneys, hence frequently leading to a rapidly progressive GN and acute renal failure. Since the initial symptoms of ANCA-associated vasculitis (AAV) vary and are often unspecific, serological determination of ANCA is an essential tool for the identification and differentiation of AAV. The most important ANCA target either proteinase 3 (PR3) which are sensitive and specific markers for Wegener's granulomatosis, or MPO which occur in microscopic polyangiitis and the Churg Strauss syndrome. The consensus on ANCA testing requires screening with indirect immunofluorescence (IIF) and confirmation in MPO- and PR3-ANCA specific assays. As a multiplexing approach, the EUROPLUS™ system combines the conventional cell substrates with single microdots of purified PR3 and MPO in one incubation field. It allows the simultaneous observation of ANCA IIF patterns on ethanol- and formalin-fixed granulocytes together with exclusion of ANA interference and the monospecific determination of MPO- and PR3-reactivity. This combination greatly supports the reading and interpretation of the ANCA IIF patterns. Additionally, the EUROPLUS™ Granulocyte Mosaic can be supplemented with microdots of GBM antigen in order to analyze potential double-positivity of ANCA and anti-GBM antibodies (see Anti-GBM antibodies in Goodpasture's disease) (Fig. 2).

A further major advance in ANCA testing is the development of an ELISA based on a novel PR3 diagnostic antigen, which consists of a mixture of human native (hn) PR3 and human cell-expressed human recombinant (hr) designer PR3, exhibiting modified N- and C-terminal signal sequences as well as an inactivated enzymatic core. This Anti-PR3-hn-hr ELISA increases the sensitivity for cytoplasmic (C)-ANCA positive AAV patients to 95 % at a specificity of 99%, thus resulting in an excellent diagnostic performance.

## ANTI-DSDNA AND ANTI-NUCLEOSOME ANTIBODIES IN LUPUS NEPHRITIS

Anti-nucleosome antibodies (ANuA) represent the first serological →

marker described in SLE which counts lupus nephritis (LN) among its many and variable manifestations. Although the prevalence of ANuA in sera from SLE patients is high, the diagnostic use of this parameter has been limited for a long time, since sera from patients with progressive systemic sclerosis (PSS) demonstrated significant positive reactions (10-68 %) with conventional ANuA test systems (1st generation). These false positive reactions were eliminated in the 2nd generation Anti-Nucleosomes ELISA: here, the antigen is based on an innovative purification protocol using sucrose density gradient centrifugation under carefully optimized NaCl conditions. This procedure results in highly purified mononucleosomes which are free from contaminating histone H1, non-histone proteins such as Scl-70, and chromatin DNA fragments. Using the 2nd generation Anti-Nucleosomes ELISA, specificity was 100 % with respect to healthy blood donors and PSS patients. In contrast, in 1st generation Anti-Nucleosomes ELISA 52 % of sera from PSS patients cross-reacted with Scl-70 due to impurities of the nucleosome preparation. Sensitivity was comparable in both assays. ANuA are particularly suitable as a prognostic indicator for SLE with renal involvement: in lupus nephritis patients requiring transplantation a considerably high prevalence (79%) was observed compared to less severe cases (18%) and SLE patients without nephritis (9%).

Anti-dsDNA antibodies are found in 60-90% of SLE patients and represent the most established marker for the disease. In order to improve the diagnostic performance of Anti-dsDNA ELISA in comparison to established test systems, an optimized test referred to as Anti-dsDNA-NcX ELISA was developed: this ELISA is based on a novel coating technology that applies the highly purified mononucleosomes used in the 2nd generation Anti-Nucleosomes ELISA as linker substance for the immobilization of dsDNA. The specific configuration of the Anti-dsDNA-NcX-ELISA avoids unspecific reactions that typically occur with conventionally used coating materials and ensures the clear and authentic presentation of the major DNA epitopes. This results not only in a strong increase in sensitivity (67%) compared to Anti-Nucleosomes ELISA (56%) and conventional anti-dsDNA ELISA (42%) at a set specificity of 98% but also in the detection of a considerable number of patient samples which are exclusively positive in the novel test system (Fig. 3).

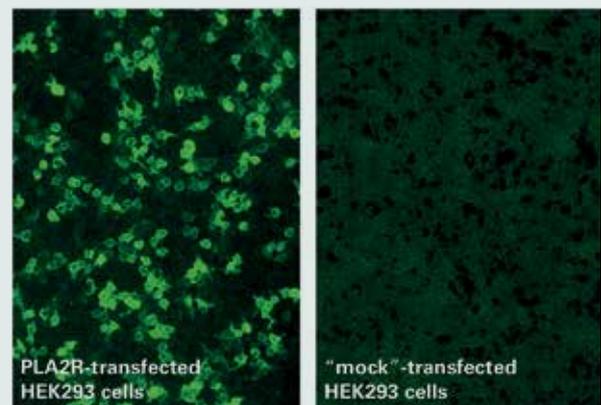
## CONCLUSION

Autoantibodies can be found in several forms of nephropathies. They can either be directed against kidney-specific autoantigens or against ubiquitous antigens as in systemic autoimmune diseases with renal manifestations. Recent developments in autoantibody diagnostics in nephrology include the identification of PLA2R and THSD7A as antigenic targets in primary membranous nephropathy, as well as considerable improvements in sensitivity, specificity and convenience of test systems for anti-dsDNA, ANuA, ANCA and anti-GBM. These advances have boosted the ease, reliability and relevance of autoantibody testing, aiding the diagnosis of autoimmune nephropathies, especially in early stages. This is crucial for the decision on interventional therapy e.g. with immunosuppressants, which can help avoid the serious and irreversible damage that occurs with disease progression. This, in turn, may circumvent the need for intensive and distressing end-stage procedures such as dialysis and transplantation – a boon for patients to the benefit of healthcare system. 📄

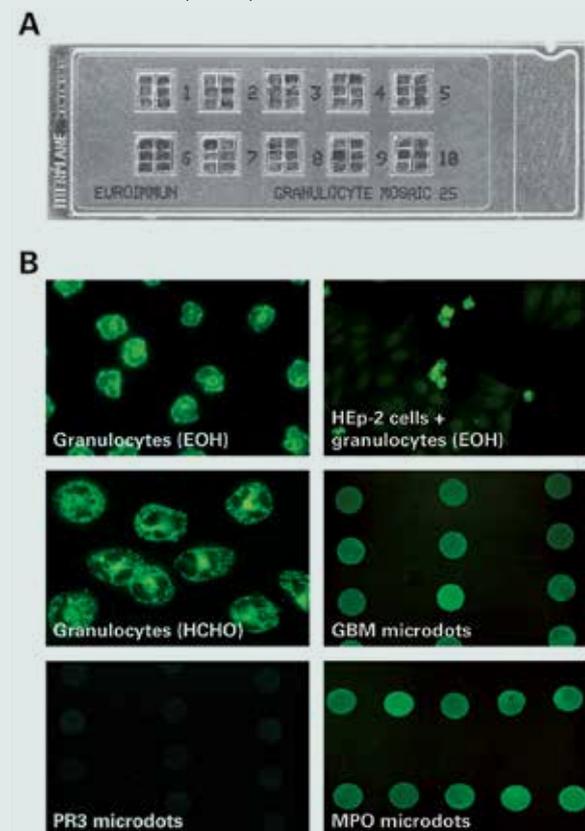
## REFERENCES

References available on request ([magazine@informa.com](mailto:magazine@informa.com))

▼ **FIGURE 1:** Positive reaction in Anti-PLA2R RC-IFA.



▼ **FIGURE 2:** EUROPLUS™ Granulocyte Mosaic: A microscope slide. B Example of BIOCHIP mosaic results (P-ANCA pattern, anti-MPO and anti-GBM positive).



▼ **FIGURE 3:** Venn-Diagramm: Anti-dsDNA-NcX ELISA detects 13 patient samples which are negative in conventional Anti-dsDNA and Anti-Nucleosomes ELISA.

