Clinical Relevance of Domain-Specific Phospholipase A$_2$ Receptor 1 Antibody Levels in Patients with Membranous Nephropathy

L. Reinhard[*], G. Zahner[1*], S. Menzel, F. Koch-Nolte, R.A.K. Stahl[§], E. Hoxha[§]

[*],[§]: equal contributions

ABSTRACT:

BACKGROUND: Antibodies against phospholipase A$_2$ receptor 1 (PLA$_2$R1) are found in 80% of patients with membranous nephropathy, and previous studies described three autoantibody-targeted PLA$_2$R1 epitope regions. Although anti-PLA$_2$R1 antibody levels are closely associated with treatment response and disease prognosis, the clinical role of epitope regions targeted by autoantibodies is unclear.

METHODS: In a prospective cohort of 150 patients with newly diagnosed PLA$_2$R1-associated membranous nephropathy, we investigated the clinical role of epitope-recognition patterns and domain-specific PLA$_2$R1 antibody levels by western blot and ELISA.

RESULTS: We identified a fourth epitope region in the CTLD8 domain of PLA$_2$R1, which was recognized by anti-PLA$_2$R1 antibodies in 24 (16.0%) patients. In all study patients, anti-PLA$_2$R1 antibodies bound both the N-terminal (CysR-FnII-CTLD1) region and the C-terminal (CTLD7-CTLD8) region of PLA$_2$R1 at study enrollment. The total anti-PLA$_2$R1 antibody levels of patients determined detection of domain-specific PLA$_2$R1 antibodies, and thereby epitope-recognition patterns. A remission of proteinuria occurred in 133 (89%) patients and was not dependent on the domain-recognition profiles. A newly developed ELISA showed that domain-specific PLA$_2$R1 antibody levels targeting CysR, CTLD1, and CTLD7 strongly correlate with the total anti-PLA$_2$R1 antibody level (Spearman's rho, 0.95, 0.64, and 0.40; $P<0.001$, $P<0.001$, and $P=0.002$, respectively) but do not predict disease outcome independently of total anti-PLA$_2$R1 antibody levels.

CONCLUSIONS: All patients with PLA$_2$R1-associated membranous nephropathy recognize at least two epitope regions in the N- and C-terminals of PLA$_2$R1 at diagnosis, contradicting the hypothesis that PLA$_2$R1 "epitope spreading" determines the prognosis of membranous nephropathy. Total anti-PLA$_2$R1 antibody levels, but not the epitope-recognition profiles at the time of diagnosis, are relevant for the clinical outcome of patients with this disease.

STATEMENT:

Our study shows for the first time that total PLA$_2$R1 antibody levels rather than antibody epitope specificity or epitope specific antibody levels are the decisive factor for treatment response and long-time prognosis in patients with membranous nephropathy. These findings are clinically highly relevant, since current evidence was inconsistent and partly suggested that immunosuppressive treatment should be tailored based on epitope specificity of antibodies, rather than on antibody levels. The combination of clinical, biomolecular and immunological expertise lead to the development of a novel sensitive ELISA based on PLA$_2$R1 domain specific Fc-fusion proteins. We performed a detailed characterization of a large prospective patient cohort provided by the UKE-based Hamburg Glomerulonephritis Registry, which is one of the largest international glomerulonephritis biobanks. Our findings

will have a direct clinical impact, facilitating the diagnosis and treatment of patients with membranous nephropathy. We demonstrate that the monitoring of the total PLA$_2$R1 antibody levels, which are already easily accessible, is sufficient to predict treatment response and long-term outcome in membranous nephropathy. On the contrary, there is currently no advantage in bringing a more complex and less robust PLA$_2$R1 epitope characterization into the clinical routine. The significance of our findings was further discussed in an Editorial accompanying our publication (Beck et al., JASN 2020; 31(1): 8-11).

BACKGROUND:

Dr. Linda Reinhard is an experienced protein biochemist and structural biologist with a special focus on protein stability and molecular recognition patterns. She works in the III. Department of Medicine in the group of Prof. Rolf A. K. Stahl and PD Dr. Elion Hoxha since 2016. Prof. Stahl has a more than 30 years lasting scientific experience in the study of the pathogenesis of membranous nephropathy. PD Dr. Hoxha is a Heisenberg fellow focusing on translational research and his work has significantly contributed to defining the clinical role of PLA$_2$R1 antibody levels in membranous nephropathy. The work was carried out in collaboration with PD Dr. Gunther Zahner (III. Medical Department) who has a broad experience in protein biochemistry as well as Prof. Friedrich Koch-Nolte and Dr. Stephan Menzel from the Department of Immunology, who are renowned experts in the field of nanobody technology. Serum samples were provided by the UKE-based Hamburg Glomerulonephritis Registry. The project was funded by the DFG (SFB1192 and Heisenberg Programme).