Our experience with anti-phospholipase A2 receptor (PLA2R) indirect immunofluorescence test

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INTRODUCTION

Idiopathic membranous nephropathy (iMN) is a common form of nephrotic syndrome. It is an antibody-mediated autoimmune glomerular disease. Autoantibodies are directed against phospholipase A2 receptor (anti-PLA2R), a transmembrane glycoprotein that is present in human glomeruli on the surface of podocytes. The autoimmune mechanism of iMN was originally described in 2009 by Beck[1]. Circulating anti-PLA2R autoantibodies bind to PLA2R, form immune complexes, activate complement and subsequently cause damage to podocytes. This process leads to broadening of the basement membrane and proteinuria[2].

Testing for anti-PLA2R is usually required:

- when nephrotic syndrome is present and/or renal biopsy can not be performed,
- to distinguish between idiopathic and secondary MN (sMN),
- for therapy monitoring.

MATERIALS AND METHODS

The method of estimation in this work was indirect immunofluorescence with PLA2R-transfected and non-transfected HEK293 cells as substrate (TITERPLAN technique with BIOCHIP slides, EUROIMMUN, Germany). Slides were prepared according to manufacturer instructions (1:10 dilution). Positive samples show characteristic cytoplasmic fluorescence of PLA2R-transfected cells.

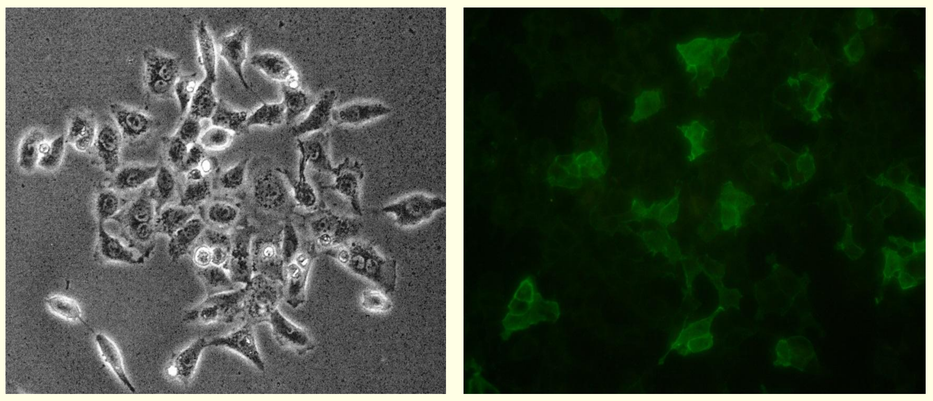

Figure 1: Hek293 cells under a light microscope (left) and fluorescently stained.(right).

A total of 234 determinations/141 patients were tested with 52 patients tested repeatedly during therapy (Fig. 2). 84 patients had iMN, 7 secondary nephropathy (sMN) and 50 other disease(s) – see Fig. 3 for diagnoses overview.

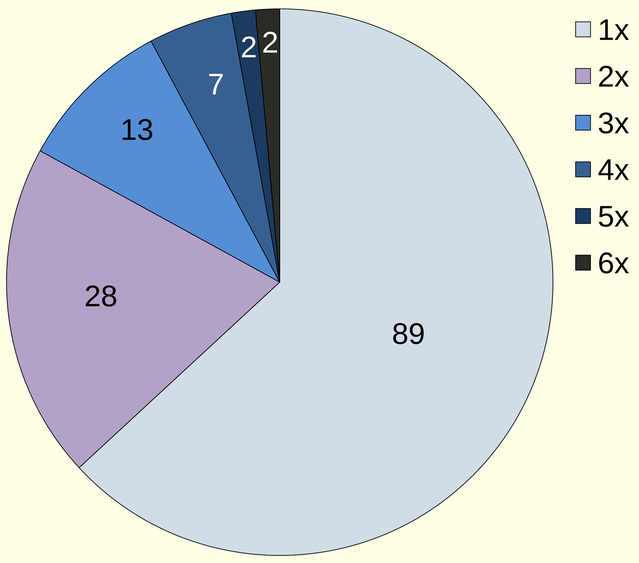

Figure 2: Patients group overview with number of tests per patient.

Patients no.	primary diagnosis	secondary diagnosis
84	iMN	-
7	sMN	overlap sy/TTP, lung tu, tonsilla tu, thyroid tu, hepatitis B, bladder tu, SLE
50	other (e.g. focal and segmental glomerulosclerosis, nephrotic syndrome (no biopsy), IgA nephropathy, SLE, minimal change disease)	-

Figure 3: Diagnoses overview for respective patient groups.

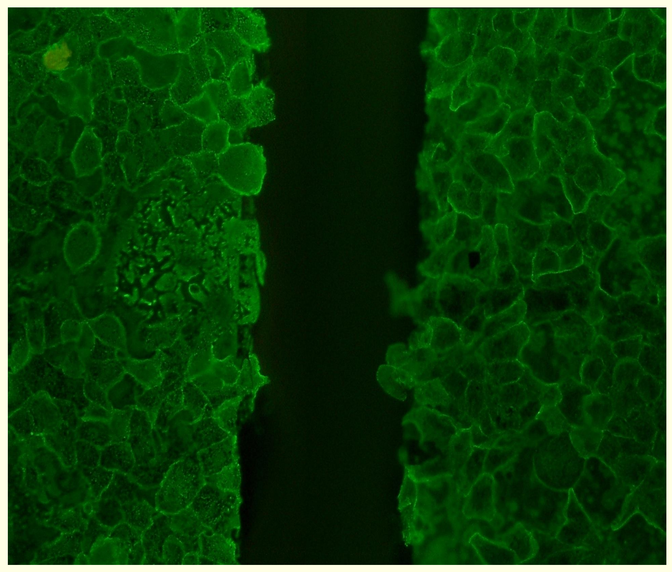

Figure 4: Atypical fluorescence pattern of non-transfected (left) and transfected (right) HEK293 cells.

A. Patient with newly diagnosed active disease who achieved remission after immunosuppression (oral cyclophosphamide + corticosteroids):				B. Patient with relapsing disease (diagnosis in 2001, relapse in 02/2012, treated with antihypertensives):				C. Patient with refractory disease course (treatment failure, progressed to end-stage renal failure):		
Date	9/2012	1/2013	3/2013	10/2011	2/2012	4/2012	7/2012	4/2012	10/2012	2/2013
PLA2R Ab	positive	negative	negative	weakly pos.	N/A	weakly pos.	negative	positive	positive	positive
Proteinuria (g/24h)	9	3	0.4	0.3	4.9	1.3	0.8	5.6	7.4	6.5
S-albumin (g/l)	22	28	43	40	40	43	46	27	32	33
S-creatinine (µmol/L)	91	86	73	123	130	165	166	228	382	603

Figure 5: Examples of patients treated for iMN repeatedly tested for aPLA2R autoantibodies during therapy.

RESULTS

Results were interpreted at the screening titer (1:10) on the following scale: negative – borderline – weakly positive – positive, see Fig. 6. From all determinations, 3 could not be microscopically determined, since transfected and non-transfected cells showed the same atypical pattern, see Fig. 4. No factor that could interfere with the fluorescence was found in these 3 samples. Primary diagnoses for these patients were primary membranous nephropathy, IgA nephropathy and chronic hidradenitis with suspected secondary amyloidosis.

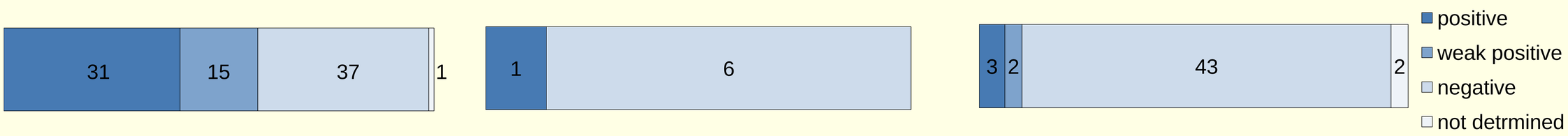

Figure 6: Results of anti-PLA2R determination in respective patient groups: iMN (left), sMN (middle), other disease (right).

On the basis of our results, the overall sensitivity and specificity of anti-PLA2R determination for iMN was found to be 55 % and 90 % respectively. Fig. 5 shows examples of patients treated for iMN that demonstrate clinical usefulness of aPLA2R test – A.) remission could be predicted before proteinuria decreases; B.) increased level of aPLA2R autoantibodies indicates relapse of the disease with proteinuria still low; C.) together with other markers confirming treatment-refractory disease.

CONCLUSION

Our experience with anti-PLA2R autoantibodies test confirmed its usefulness for clinical practice. It is very helpful for differential diagnostic purposes, therapy monitoring, where it helps to predict relapse of the disease or treatment response before changes of proteinuria are detected. Moreover, when a patient with membranous nephropathy is tested anti-PLA2R negative, it is necessary to look for causes of MN other than autoimmune, often paraneoplastic.

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2. Glassock RJ. The pathogenesis of membranous nephropathy: evolution and revolution. Curr Opin Nephrol Hypertens. 2012 May;21(3):235-42.