

Value of immunofluorescence-mediated detection of Ig, C1q, C3, and FRA for the identification and diagnosis of atypical membranous nephropathy

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Abstract. – OBJECTIVE: The present study was to investigate the value of immunofluorescence-mediated detection of Ig, C1q, C3, and FRA for the identification and diagnosis of atypical membranous nephropathy (AMN).

PATIENTS AND METHODS: Fifty-five patients with AMN and 135 patients with idiopathic membranous nephropathy (IMN) diagnosed by renal biopsy in our hospital were consecutively selected. The positive expressions of Ig, C1q, C3, and FRA by immunofluorescence were analyzed.

RESULTS: We compared the levels of blood urea nitrogen, creatinine, cystatin C, and 24 h urine protein, and the levels of serum IgA, IgG, IgM, and C3. The differences were not significant ($p>0.05$). Proportionate increases in glomerular spiky projection formations in patients with AMN were observed by light microscopy, without observation of other pathologic changes. By immunofluorescence, AMN patients showed higher positive rates of deposition of IgA, IgM, C1q, and FRA compared with IMN patients. Comparison of the positive rates of deposition of IgG and C3 showed no differences. By electron microscopy, AMN patients showed higher percentages of mesangial cell and mesangial matrix proliferation. Deposition of electron dense granules was mostly found in subepithelium, inside basement membranes, and in the mesangial area. The comparisons between the two groups showed no differences.

CONCLUSIONS: Immunofluorescence-mediated detection of Ig, C1q, C3, and FRA have important application value for the identification and diagnosis of AMN.

Key Words:

Immunofluorescence, Ig, C1q, C3, FRA, Atypical membranous nephropathy.

Introduction

The main pathologic changes of membranous nephropathy are thickening of the glomerular

basement membrane combined with deposition of immune complexes under epithelial cells, and fine granular deposits of IgG and C3 along glomerular capillary walls, as determined by immunofluorescence microscopy¹. The rate of morbidity associated with nephrotic syndrome in clinical practice is about 30-65%. Patients of all ages can suffer from the disease with various clinical symptoms and varied treatment effects²⁻⁴. The causes of membranous nephropathy are divided into idiopathic membranous nephropathy (IMN) and secondary membranous nephropathy (SMN). Furthermore, some patients have pathological manifestations that are similar to secondary membranous nephropathy, but have no confirmed basic diseases, which is known as atypical membranous nephropathy (AMN)⁵⁻⁷. There are relatively few clinical and pathological studies on AMN. The aim of the present work was to investigate the value of immunofluorescence-mediated detection of Ig, C1q, C3, and FRA for the identification and diagnosis of AMN.

Patients and Methods

Patients

Fifty-five patients with AMN and 135 patients with IMN diagnosed by renal biopsy in our hospital from January 2013 to October 2016 were retrospectively analyzed. The informed consent was obtained from all the patients. The investigation was approved by the Ethical Committee of Tianjin Hospital. We excluded cases of glomerulonephritis, renal dysfunction, pyelonephritis, other immune diseases, secondary membranous nephropathy, and recent treatment history with hormones and immunosuppressive agents. The

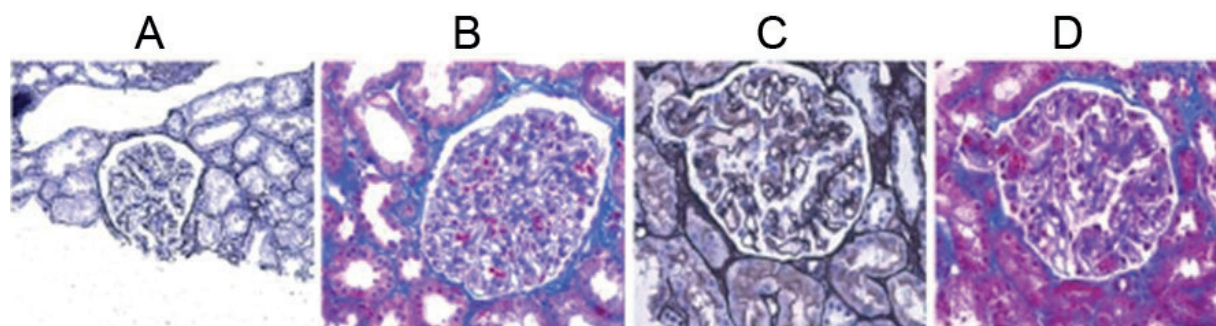


Figure 1. Imaging analysis of light microscopic report of renal pathology (**A**, IMN, middle amplification, PASM staining; **B**, IMN, high amplification, Masson staining; **C**, AMN, high amplification, PASM staining; **D**, AMN, high amplification, Masson staining). **A-B**, Renal biopsy showed nine glomeruli, mild diffuse proliferation of glomerular mesangial cells and mesangial matrix, and the deposition of fuchsinophilic protein in the mesangial area; one glomerulus showed segmental sclerosis at the urinary pole. Renal tubular epithelial cell vacuolar and granular degeneration, atrophic lesions, and local lymphocytic and monocyte infiltration combined with fibrosis were found in the mesangial matrix. Wall thickening of arterioles was found. Immunofluorescence: four glomeruli were shown, and were IgA (+++), IgG (-), IgM (++), Clq (-), C3 (++), and FRA (-). There was deposition of conglomerations along the mesangial area. Electron microscopy showed FSGS accompanied with IgA deposition (details were listed in the report of electron microscopic examination). According to the results of electron microscopy, patients were diagnosed with focal segmental glomerulosclerosis (apical type) accompanied with IgA deposition. **C-D**, Renal biopsy showed 19 glomeruli, mild diffuse proliferation of glomerular mesangial cells and mesangial matrix, basement membrane vacuolar degeneration, and podocyte proliferation. One glomerulus showed segmental sclerosis at the urinary pole. Renal tubular epithelial cell vacuolar and granular degeneration, atrophic lesions, and local lymphocytic and monocyte infiltration combined with fibrosis were observed. Wall thickening of arterioles was found. Immunofluorescence: six glomeruli were shown, and were IgA (-), IgG (-), IgM (-), Clq (++), C3 (+), and FRA (++). There was deposition of conglomerations and granular deposition along the mesangial area and capillaries. Electron microscopy showed AMN (details were listed in the report of electron microscopic examination). The results of electron microscopic analysis showed AMN accompanied with focal segmental glomerulosclerosis (apical type).

baseline parameters of the two groups were comparable (Table I).

Research Methods

Biopsies were analyzed by the Department of Kidney Pathology. Analysis included the number of glomeruli and glomerular spiky projection by light microscopy, detection of IgA, IgM, Clq, and FRA by immunofluorescence microscopy, the number of mesangial cells and mesangial matrix proliferation, deposition of electron dense granules by electron microscopy, periodic acid-silver methenamine (PASM) staining under the light microscope (medium or high amplification), and Masson staining (medium amplification) (Figure 1).

We analyzed the levels of blood urea nitrogen, creatinine, cystatin C, and 24 h urine protein. Furthermore, we analyzed the levels of IgA, IgG, IgM, and C3 in serum and by immunofluorescence microscopy (positive expression of IgA, IgG, IgM, Clq, C3, and FRA), and electron microscopy.

Statistical Analysis

SPSS20.0 software (SPSS, IBM Corp, Armonk, NY, USA) was used for data analysis. Numerical data are presented as mean \pm standard deviation.

Comparisons were by independent-samples *t*-test. Categorical data are presented as case number or percentage (%). The comparisons among the groups were made by χ^2 -test; $p < 0.05$ was considered statistically significant.

Results

Comparison of the Levels of Blood Urea Nitrogen, Creatinine, Cystatin C, and 24 h Urine Protein

The comparison of the levels of blood urea nitrogen, creatinine, cystatin C, and 24 h urine protein between the two groups showed no significant differences ($p > 0.05$) (Table II).

Comparison of Serum Levels of IgA, IgG, IgM, and C3

The comparison of the serum levels of IgA, IgG, IgM, and C3 between the two groups showed no significant differences ($p > 0.05$) (Table III).

Comparison of the Results of Light Microscopy

A proportionate increase of glomerular spiky projection formations in patients with

Table I. Comparison of baseline parameters of the two groups.

Group	Male/ female	Age (y)	Disease course (months)	Hypertension [case (%)]	Edema [case (%)]	Hyperlipemia [case (%)]	Hypoproteinemia [case (%)]
AMN (n=55)	30/25	55.8±12.6	3.8±0.7	40 (72.7)	36 (65.5)	33 (60.0)	29 (52.7)
IMN (n=135)	72/63	54.9±13.3	3.9±0.8	105 (77.8)	98 (72.6)	90 (66.7)	82 (60.7)
t/χ^2	0.023	0.252	0.186	0.551	0.958	0.761	1.033
p	0.879	0.867	0.923	0.458	0.328	0.383	0.309

Table II. Comparison of the levels of blood urea nitrogen, creatinine, cystatin C, and 24 h urine protein.

Group	Urea nitrogen (mmol/l)	Creatinine (μ mol/l)	Cystatin C (mg/l)	24 h urine protein (g)
AMN	5.3±1.4	123.5±34.7	2.5±0.8	6.6±1.5
IMN	5.5±1.7	132.6±42.5	2.3±0.9	6.9±1.7
t	0.152	0.356	0.202	0.325
p	0.768	0.764	0.854	0.648

Table III. Comparison of serum levels of IgA, IgG, IgM, and C3 (g/l).

Group	IgA	IgG	IgM	C3
AMN	3.2±1.3	12.8±3.3	3.4±0.9	2.6±0.7
IMN	3.4±1.4	13.3±3.5	3.2±0.7	2.7±0.9
t	0.085	0.285	0.286	0.186
p	0.953	0.864	0.847	0.897

Table IV. Comparison of the results of light microscopy.

Group	Glomerular		Renal Tubular		Renal vessel abnormality		
	Glomerulosclerosis	Spiky projection	Inflammatory cell infiltration	Matrix Atrophy	Inflammatory cell infiltration	Fibrosis	
AMN (n=55)	32 (58.2)	25 (45.5)	15 (27.3)	33 (60.0)	36 (65.5)	32 (58.2)	22 (40.0)
IMN (n=135)	70 (51.9)	40 (29.6)	41 (30.4)	79 (58.5)	94 (69.6)	96 (71.1)	60 (44.4)
χ^2	0.630	4.348	0.180	0.035	0.315	2.972	0.315
p	0.427	0.037	0.671	0.851	0.574	0.085	0.575

Table V. Comparison of the results of immunofluorescence.

Group	IgA	IgM	IgG	C1q	C3	FRA
AMN (n=55)	31 (56.4)	32 (58.2)	50 (90.9)	43 (78.2)	46 (83.6)	28 (50.9)
IMN (n=135)	41 (30.4)	55 (40.7)	129 (95.6)	61 (45.2)	120 (88.9)	39 (28.9)
χ^2	11.219	4.789	1.547	17.173	0.977	8.301
p	0.001	0.029	0.214	0.000	0.323	0.004

AMN was observed by light microscopy, without observation of other pathologic changes (Table IV).

Comparison of the Results of Immunofluorescence

Patients with AMN showed higher rates of positive deposition of IgA, IgM, C1q, and FRA

compared with patients with IMN. There were no differences in the comparison of the positive rates of deposition of IgG or C3 (Table V).

Comparison of the Results of Electron Microscopy

In both patients with AMN and IMN, we observed thickening of glomerular basement mem-

Table VI. Comparison of the results of electron microscopy.

Group	Mesangial cell and mesangial matrix proliferation	Deposition of electron dense granules
AMN (n=55)	20 (36.4)	43 (78.2)
IMN (n=135)	28 (20.7)	109 (80.7)
χ^2	5.052	0.160
<i>p</i>	0.025	0.689

branes and diffuse foot process fusion, without specific lesions of renal tubules or renal interstitium. Patients showed higher percentages of mesangial cell and mesangial matrix proliferation. Deposition of electron dense granules was mostly observed in the subepithelium, inside basement membranes, and in the mesangial area. Comparisons between the two groups showed no differences (Table VI).

Discussion

There is currently no large-scale research on AMN. The exact definition and classification of AMN is still disputed. Some scholars believe it should be classified as IMN⁸, while others consider AMN as a form of secondary membranous nephropathy without definite cause⁹. However, AMN is rarely considered as a form of independent membranous nephropathy.

According to our study, there were no differences in sex, age, disease course, or clinical manifestations between patients with AMN and IMN. A previous research¹⁰ indicated that AMN is more often accompanied with hematuria compared with IMN, which may be correlated with the fact that AMN is manifested by mesangial cell and mesangial matrix proliferation, and increases in immunoglobulin deposition, such as IgA. We compared the levels of blood urea nitrogen, creatinine, cystatin C, and 24 h urine protein, and the levels of serum IgA, IgG, IgM, and C3. There were no significant differences, indicating that the clinical and laboratory differentiation between the two groups is relatively difficult^{11,12}. There is research suggesting that compared with IMN, AMN is associated with increased levels of several inflammatory factors, often secondary to infection, malignant tumors, or autoimmune diseases that involve the kidney¹³. Proportionate increases of glomerular spiky projection formations in patients with AMN were observed by light microscopy, without observation of other pathologic

changes. Patients with AMN showed higher positive rates of deposition of IgA, IgM, C1q, and FRA compared with patients with IMN. Comparison of the positive rates of deposition of IgG and C3 showed no differences. A previous research¹⁴ indicated that the differences in the observations by immunofluorescence between AMN and SMN caused by membranous nephropathy related to systemic lupus erythematosus and hepatitis B were not significant. It was also noted that AMN may be SMN that has no definite cause at different stages. AMN patients showed higher percentages of mesangial cell and mesangial matrix proliferation. Deposition of electron dense granules was mostly found in subepithelium, inside basement membranes, and in the mesangial area. Careful observation by light microscopy, immunofluorescence, and electron microscopy can improve the diagnostic accuracy of AMN^{15,16}.

The pathological characteristics of IMN include the fine granular deposition of IgG and C3 along glomerular capillary walls, as determined by immunofluorescence. Additionally, the deposition of IgM, IgE, and C1q can be observed. The amount of deposition changes with the disease course¹⁷. The amount of deposition is small at the early stage, and increases with disease progression, while at the advanced stage, it decreases again¹⁸. Diagnosis of membranous nephropathy relies primarily on renal biopsy. There are many studies aiming at identifying highly sensitive and specific serological markers to replace renal biopsy¹⁹. The positive expression rates of angiotensin-like protein 4 and PLA2R have been shown to be as high as 70-85% in IMN^{20,21}. Furthermore, the detection of IgG subtypes by immunohistochemistry contributes to the differentiation of AMN and IMN²².

Conclusions

The immunofluorescence-mediated detection of Ig, C1q, C3, and FRA has important applica-

tion value for the identification and diagnosis of AMN.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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