Clin Exp Nephrol (2008) 12:421–431
DOI 10.1007/s10157-008-0104-z

ORIGINAL ARTICLE

Special issue
Podocytic infolding glomerulopathy: a proposed new disease entity

Proposal of podocytic infolding glomerulopathy as a new disease entity: a review of 25 cases from nationwide research in Japan

Kensuke Joh · Takashi Taguchi · Hidekazu Shigematsu · Yutaka Kobayashi · Hiroshi Sato · Shinichi Nishi · Ritsuko Katafuchi · Shinsuke Nomura · Yoshihide Fujigaki · Yasunori Utsunomiya · Hitoshi Sugiyama · Takao Saito · Hirofumi Makino

Received: 21 July 2008 / Accepted: 2 September 2008 / Published online: 18 November 2008

Abstract

Background A rare and peculiar glomerulopathy has begun to be recognized in Japan. The Japanese Society of Nephrology has established a research working group and has collected cases from all over Japan in an attempt to understand the complete spectrum of this glomerulopathy.

Method The diagnostic criterion, which was needed to collect the cases, was proposed as a glomerulopathy showing microspheres or microtubular structures or both associated with podocytic infolding into the glomerular basement membrane (GBM) on electron microscopy. The lesion shows a non-argentaffin hole in the GBM with periodic acid methenamine silver staining and is similar to membranous glomerulonephritis.

K. Joh (✉)
Division of Renal Pathology, Clinical Research Center,
Chiba-East National Hospital, 673 Nitona-cho, Chuo-ku,
Chiba 260-8712, Japan
e-mail: johken@cehprinet.com

T. Taguchi
Department of Pathology,
Nagasaki University Graduate School of Biomedical Sciences,
Nagasaki, Japan

H. Shigematsu
Department of Histopathology,
Shinshu University School of Medicine,
Matsumoto, Japan

Y. Kobayashi
Division of Nephrology, Akebono Hospital, Tokyo, Japan

H. Sato
Department of Nephrology, Hypertension, Endocrinology,
Tohoku University Graduate School of Medicine,
Sendai, Japan

S. Nishi
Division of Clinical Nephrology and Rheumatology,
Graduate School of Medical and Dental Sciences,
Niigata University, Niigata, Japan

R. Katafuchi
Division of Internal Medicine,
National Fukuoka-Higashi Medical Center,
Fukuoka, Japan

S. Nomura
Department of Cardiology and Nephrology,
Mie University Graduate School of Medicine, Mie, Japan

Y. Fujigaki
The First Department of Medicine,
Hamamatsu University School of Medicine,
Hamamatsu, Japan

Y. Utsunomiya
Division of Kidney and Hypertension,
The Jikei University School of Medicine, Tokyo, Japan

H. Sugiyama · H. Makino
Department of Medicine and Clinical Science,
Okayama University Graduate School of Medicine,
Dentistry and Pharmaceutical Sciences, Okayama, Japan

T. Saito
Division of Nephrology and Rheumatology,
Department of Internal Medicine,
Fukuoka University School of Medicine,
Fukuoka, Japan

Results Twenty-five cases were collected from 17 institutions. Patients were 20–69 years old (19 women, 6 men). Seventeen patients also had collagen diseases such as lupus nephritis and Sjögren's syndrome. All patients had proteinuria. Proteinuria showed a remission in 15 of 23 patients within 12 months, but proteinuria remained higher than 1.0 g/day in five patients despite different types of therapy. Podocytic infolding including microspheres showed either positive or negative staining for immunoglobulins. Cluster formation of microspheres was found in 4 of 17 patients with collagen disease, and in five out eight patients without collagen disease. Electron-dense deposits in the GBM were also found in 6 of 17 patients with collagen disease but were not found in eight patients without collagen disease.

Conclusion Some patients might have a subtype of lupus nephritis, class V, or membranous glomerulonephritis. However, we propose a new disease entity, podocytic infolding glomerulopathy, as a common basis of all 25 patients, because we suspect that microspheres or microtubular structures or both can be derived from podocytic infolding.

Keywords Renal biopsy · Glomerulonephritis · Podocytic infolding · Electron microscopy · Microspheres

Introduction

Recently, we have begun to recognize a rare and peculiar glomerulopathy, in which microspheres or microtubular structure or both are associated with infolding of cytoplasmic processes of podocytes into the glomerular basement membrane (GBM). This type of glomerulopathy is not included in the World Health Organization's classification of glomerular diseases [1]. We have, as yet, no idea, whether this lesion indicates a new disease entity or a transient morphological finding of a well-known disease. To more clearly understand the clinical and pathological profile, treatment, and prognosis of this glomerulopathy, we need to analyze many cases. However, it was difficult to accumulate many cases of this kind of glomerulopathy in a single institution, because the glomerulopathy is extremely rare. Therefore, the Japanese Society of Nephrology has established a research working group and has collected cases from all over Japan in an attempt to understand the complete spectrum of this glomerulopathy. As a result, 25 cases have been collected from 17 institutions across Japan. We report a summary of the findings and discuss whether a new disease entity should be proposed.

Collection of the patients

The patients were distinguished with ultrastructural studies. The following electron-microscopic finding was proposed as a common tentative criterion, which was needed to collect the cases. On electron microscopy, microspheres or microtubular structures or both are found in association with podocytic infolding into the GBM (Figs. 1, 2). The lesion was designated as podocytic infolding glomerulopathy (PIG). PIG shows a non-argentaffin hole (bubbling or stipple formation) in association with an occasional spike formation on periodic acid methenamine silver (PAM)

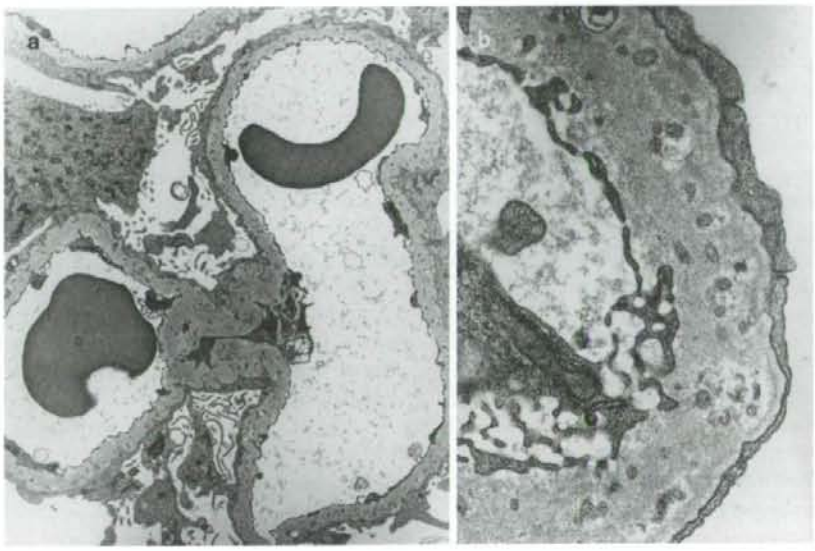


Fig. 1 **a** Electron micrograph of case 19 shows global and diffuse distribution of microstructures in the GBM (×3,000). **b** The microstructures show microspheres measuring 50–150 nm in diameter (×15,000)

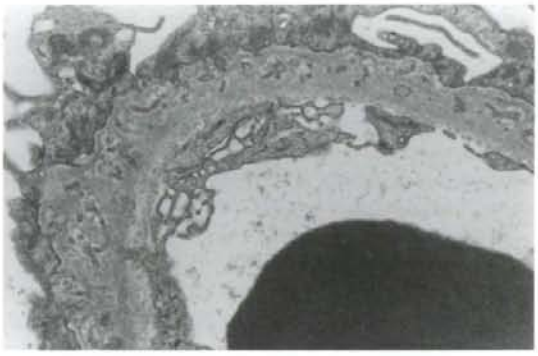


Fig. 2 Electron micrograph of case 3 shows microtubular structures with a diffuse distribution measuring 50–100 nm in width (×10,000)

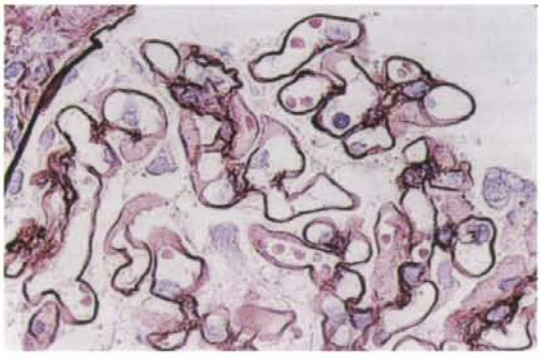


Fig. 3 Light microscopy of case 19 shows a non-argentaffin hole (bubbling or stipple formation) in the GBM. Spike formation was not remarkable (PAM stain)

staining (Fig. 3). Therefore, the light-microscopic findings are similar to those of membranous glomerulonephritis. On electron microscopy, cytoplasmic processes of podocytes infold into the GBM, which involves thickening of the lamina densa. At the end of an infolded cytoplasmic process of a podocyte, microspheres or microtubular structures or both are found (Fig. 4). These microstructures measuring 50–150 nm have a trilaminar limiting membrane structure, which is known as a unit membrane and may be equivalent to a cytoplasmic membrane (Fig. 5). Microspheres can show cluster formation (Fig. 6).

We can classify the podocytic infolding into two categories, such as primary infolding and microstructure in the GBM, by which the finding of podocytic infolding can be more clearly understood. Primary infolding is infolding of the podocytic cytoplasmic process into the GBM. Microstructures in the GBM including microspheres or microtubular structures or both are budding from the primary infolding, which is seen in the GBM. With the combination of primary infolding and microstructures in the GBM, PIG is divided into three types: type A with only primary podocytic infolding, type B with both primary podocytic infolding and microstructures in the GBM, and type C with only microstructures in the GBM (Fig. 7). We can recognize the microstructures in the GBM (type C) as bubbling formation on PAM staining, whereas primary podocytic infolding (type A) corresponds to spike formation on PAM staining.

With these diagnostic criteria for PIG, 25 cases were collected from 17 Japanese institutions. Five cases (case 1, 11, 14, 15, and 17) had previously been reported by Sato et al. [2]. The remaining cases, including case 2 [3], case 3 [3], case 4 [4], case 5 [5], case 6 [6], case 7 [6], case 8 [7], case 9 [8], case 10 [9], case 12 [10], case 13 [11], case 16 [3], case 18 [12], case 19 [13], case 20 [14], case 21 [15], case 22 [16], case 23 [17], case 24 [18], and case 25 [19], correspond to each reference which has been published in this special issue.

The clinical findings analyzed issues included age, sex, grade of proteinuria (g/day) at the time of renal biopsy and at the end of observation, hematuria, serum creatinine level at the time of renal biopsy, blood pressure, treatment, and a concomitant diseases.

The morphological findings analyzed included light-microscopic diagnoses, presence of podocytic infolding, microspheres, clusters of microspheres, microtubules, electron dense deposits in the GBM, GBM thickening, and results of immunostaining (granular pattern at the glomerular peripheral loops).

Results

Clinical profile (Table 1)

The patients ranged in age from 20 to 69 years. The greatest number of patients was in the fourth decade (Fig. 8). Nineteen patients were women, and 6 patients were men. All 25 patients showed proteinuria (<1.0 g/day in six cases, 1.0–2.9 g/day in 13 cases, >3.0 g/day in six cases), and six patients had nephrotic syndrome. Only three patients had microhematuria (more than five erythrocytes per high-power field on light microscopy of urine sediments). Hypertension (systolic blood pressure > 140 mmHg or diastolic blood pressure > 90 mmHg) was present in 7 of 25 patients. Serial measurement of urinary protein levels in 22 patients showed that proteinuria remitted within 12 months in 15 patients and that proteinuria remained higher than 1.0 g/day during the observation period in five patients. Serum creatinine levels were within the normal range in 21 of 25 patients and the remaining four patients showed increased level of serum creatinine levels greater

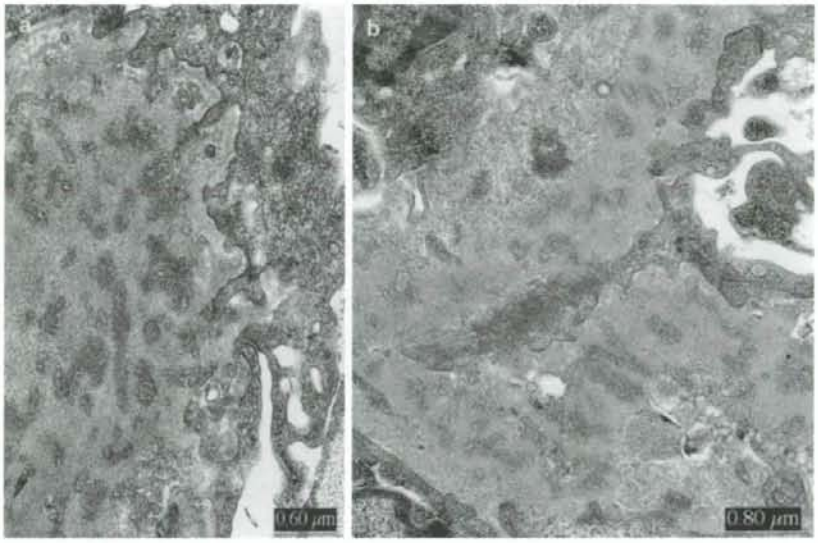


Fig. 4 **a** Electron micrograph of case 21 shows cytoplasmic processes of podocytes infolded into the GBM. At the end of an infolded cytoplasmic process of podocyte, microspheres or microtubular structures or both were found. **b** The infolded cytoplasmic process of a podocyte went through the middle layer of the GBM, which was accompanied by thickening of the lamina densa (×10,000)

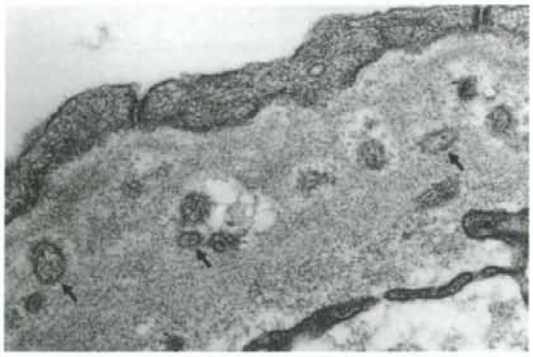


Fig. 5 The microspheres of case 19 measuring 50–150 nm in diameter have a trilaminar limiting membrane structure, which is known as a unit membrane (×30,000)

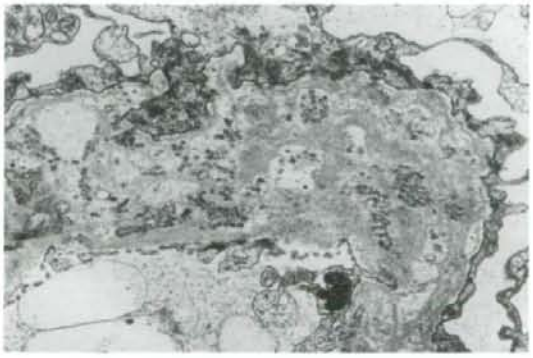


Fig. 6 The microspheres of case 23 measuring 40–60 nm in diameter showed a cluster formation (×20,000)

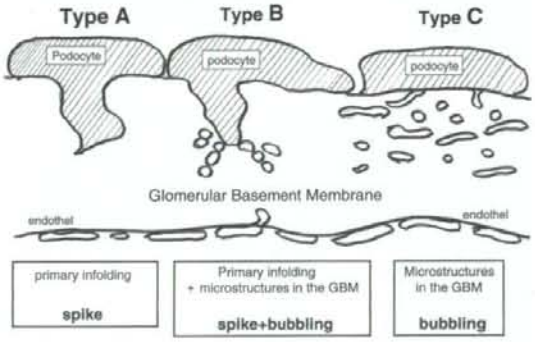


Fig. 7 A diagram of primary podocytic infolding and microstructures in the GBM. Podocytic infolding glomerulopathy was divided into three types: type A with only primary podocytic infolding, type B with both primary and microstructures in the GBM, and type C with only microstructures in the GBM

than 1.4 mg/dl, all of whom had hypertension. Prednisolone was administered to 18 patients, three of whom underwent steroid pulse therapy, and immunosuppressants such as cyclosporine A and mycophenolate mofetil were additionally used in three patients. The patients treated with prednisolone included 15 of the 17 patients with collagen disease and three of the eight patients without collagen disease. In 12 of 18 patients, proteinuria decreases with steroid administration. Prednisolone was not used in seven patients, of whom four received no treatment and three received an angiotensin II receptor antagonist or diuretics or both.

Concomitant diseases included systemic lupus erythematosus (SLE) in 14 patients, which was combined with

Table 1 Clinical profile of PIG (25 cases)

Case no.	Age (years) sex	Proteinuria		Hematuria (HPF)	Renal function (mg/dl)	Blood pressure (mmHg)	Treatment	Concomitant diseases
		Initial (g/day)	Final (g/day)					
Case 1 [2]	31 M	0.5	Remission	<1	Cr = 1.9	154/99	PSL, 20 mg/day	SLE
Case 2 [3]	37 F	1	0.2 after 6 months	<1	Cr = 1.2	124/71	PSL, 20 mg, MMF	SLE
Case 3 [3]	40 F	1.5	ND		Cr = 0.5	104/70	PSL	SLE
Case 4 [4]	30 F	1.6	0.5 after 1 month	5 < 9	Cr = 0.5	110/72	PSL (pulse × 3)	SLE
Case 5 [5]	61 F	1.7	1.6 after 7 years	<1	Cr = 0.9	142/82	PSL, 40 mg, CyA	SLE, Takayasu's arteritis
Case 6 [6]	29 F	1.6	3.8 after 5 years	<4	Cr = 0.7, Cr = 0.73 after 5 years	132/78	PSL, 20 mg/day	SLE, hydronephrosis due to lupus cystitis
Case 7 [6]	46 F	0.6	0.7 after 2 years	<4	Cr = 0.5, Cr = 0.42 after 2 years	132/74	PSL, 15 mg/day	SLE, hydronephrosis due to lupus cystitis
Case 8 [7]	27 F	2.7	0.4 after 6 months	1 < 3	Cr = 0.4	120/70	PSL, 30 mg, MMF	SLE
Case 9 [8]	53 M	NS 3.1	Remission after 8 months	<100	Cr = 0.9	150/82	PSL, 30 mg	SLE, bilateral ureteral stone
Case 10 [9]	23 F	1.8	Remission after 5 years	3+	Cr = 0.5	130/70	PSL, 40 mg	SLE
Case 11 [2]	31 F	0.5	Remission	<1	Cr = 0.9	100/60	PSL, 20 mg/day	SLE
Case 12 [10]	24 F	NS 6.0	0.15 after 9 weeks	<4	Cr = 0.6	114/70	PSL (pulse × 3)	SLE, Sjögren's synd
Case 13 [11]	49 M	2.2	Remission after 9 years	<1	Cr = 1.1	140/90	ARB, PSL 40 mg	PBC, Sjögren's synd, cystitis, finally SLE
Case 14 [2]	20 F	1.4	0.3	<1	Cr = 1.4	140/80	np	Lupus-like (3 items)
Case 15 [2]	47 F	1.3	0.9	<1	Cr = 0.6	131/81	ARB, 20 mg/day	RA, Sjögren's synd
Case 16 [3]	51 F	NS 3.7	Remission after 1 month	<1	Cr = 0.6	120/65	PSL, 40 mg	Sjögren's synd
Case 17 [2]	30 F	0.3		<1	Cr = 0.9	110/60	PSL, 15 mg/day	MCTD
Case 18 [12]	54 F	NS 6.0	2.0 after 3 months	<1	Cr = 2.5	160/100	np	VUR with bilateral hydronephrosis, Basedow's disease
Case 19 [13]	57 F	0.32	0.23 after 2 months	<1	Cr = 0.6	101/65	np	Hypothyroidism, chronic thyroiditis
Case 20 [14]	45 M	2.61	1.0 after 2 months	<1	Cr = 0.7	118/75	PSL, 50 mg/day	
Case 21 [15]	42 F	NS 7.5	4.8 after 1 month	3+	Cr = 0.77	120/85	ARB, diuretics	Ovarian mature teratoma
Case 22 [16]	69 F	1.6	0.8 g/day after 1 month	<4	Cr = 0.9	145/80	np	np
Case 23 [17]	46 M	NS 4.0	2	<1	Cr = 1.2, Cr = 1.6 after 9 years	130/86	Diuretics	HBVsAg (+)
Case 24 [18]	59 M	0.6	ND	<1	Cr = 5.1, Cr = 4.8 after 0.5 month	140/86	PSL (pulse × 3)	Tumor lysis syndrome
Case 25 [19]	45 F	1.5	Remission after 1 year	<1	Cr = 0.8	108/80	PSL, 30 mg	np

M male, *F* female, *NS* nephrotic syndrome, *HPF* high-power field, *Cr* serum creatinine, *PSL* prednisolone, *MMF* mycophenolate mofetil, *CyA* Cyclosporine A, *ARB* angiotensin II receptor antagonist, *ND* not done, *np* not particular, *SLE* systemic lupus erythematosus, *RA* rheumatoid arthritis, *MCTD* mixed connective- tissue disease, *VUR* vesicoureteral reflux

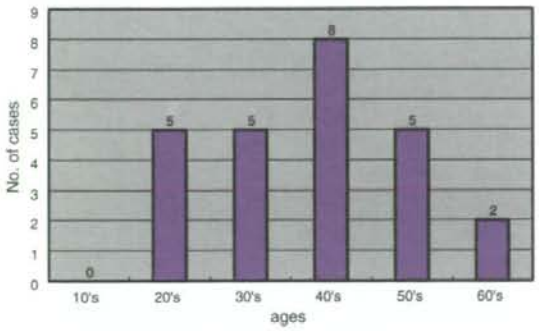


Fig. 8 Patients ranged in age from 20 to 69 years. The greatest number of patients was in the fourth decade

Sjögren's syndrome, hydronephrosis, and Takayasu's disease in two, three, and one patient, respectively, and Sjögren's syndrome in four patients, which was combined with SLE, primary biliary cirrhosis, and rheumatoid arthritis in two, one, and one patient, respectively. Mixed connective-tissue disease (MCTD), hypothyroidism due to chronic thyroiditis, hepatitis B virus infection, ovarian mature teratoma, and vesicoureteral reflux (VUR) associated with bilateral hydronephrosis and Basedow's disease were each present in one patient.

Therefore, the 25 patients can be divided into a group of 17 patients, in whom PIG was associated with a collagen disease (lupus nephritis, Sjögren's syndrome, rheumatoid arthritis, or MCTD), and eight patients, in whom PIG was associated with non-collagen disease (chronic thyroiditis, hepatitis B virus infection, ovarian mature teratoma, or VUR with Basedow's disease).

Pathological profile (Table 2)

Tentative light-microscopic diagnoses must be made without using the terminology of PIG. The diagnoses of a primary glomerulonephritis included membranous nephropathy (stage 2–3) in four patients and focal segmental glomerulosclerosis (FSGS) with bubbling in the GBM in four patients. The diagnoses of a secondary glomerulonephritis included lupus nephritis in 12 patients (class I in one patient, class II in four patients, and class V in seven patients). In four patients who had clinical diagnoses of Sjögren's syndrome, light-microscopic diagnoses included minor glomerular abnormality (MGA) in three patients and membranoproliferative glomerulonephritis (MPGN) type 3 in one patient. One patient of MCTD showed MGA.

On electron microscopy, podocytic infolding was seen in all 25 patients, of whom four patients (patients 16, 19, and 20, and 21) showed mild primary podocytic infolding but prominent microstructures in the GBM. Microspheres were present in 24 of 25 patients, of whom nine patients showed cluster of microspheres. Clusters of microspheres were found in four of 17 patients with collagen disease and in five of eight patients without collagen disease. Microtubular structures in the GBM were present in 14 of 25 patients, of whom 13 patients also showed microspheres. Thickening of the GBM, especially in the lamina densa, was seen in 24 of 25 patients. In patients with collagen disease-associated PIG, amorphous electron-dense deposits suggesting immune complex deposition as seen in membranous glomerulonephritis, were found in the mesangial area in 17 patients. However, in addition to the deposition in the mesangial area, electron-dense deposits were also found in the GBM co-localized with podocytic infolding in six of 17 patients. On the other hand, in patients with collagen disease-irrelevant PIG, electron-dense deposits could not be found in the GBM nor in the mesangial area.

On immunological studies, immunoglobulins and complements were all negative along the glomerular capillary loops in seven patients, including five of 17 patients with collagen disease-associated PIG and two of eight patients with collagen disease-irrelevant PIG. Only IgG was positive in six patients, including one patient who showed hepatitis B virus surface antigen (HBVs). Four patients were positive for IgG, IgA, C3, and C1q; 2 for IgG, IgA, IgM, and C3; 2 for IgG, IgM, and C1q, 1 for IgG, IgA, and C3, and 2 for IgG and IgA, or IgM or both. IgM only was positive in one patient. There was no apparent difference in the mode of immunoglobulin deposition between collagen disease-associated PIG and collagen disease-irrelevant PIG. Podocytic infolding including microstructures in the GBM showed either positive or negative staining for immunoglobulins.

Discussion

Twenty-five patients were divided into a group with collagen disease-associated PIG and a group with collagen disease-irrelevant PIG. In the former group, differentiation from lupus nephritis, class V should be discussed, because 14 of 17 patients had SLE. In this group, in addition to the electron-dense deposition in the mesangial area, electron-dense deposits were also found in the GBM in six of 17 patients, whereas no electron-dense deposits were found in patients with collagen disease-irrelevant PIG. Electron-dense deposits are supposedly positive for immunoglobulins and complement. It was true that the patients who showed electron-dense deposits in the GBM (patients 4, 8, 10, 11, 12, and 15), showed IgG or other immunoglobulins or both and complement on the glomerular peripheral loops. However, the patients who showed no dense

Table 2 Pathological profile of PIG (25 cases)

Case no.	Age (years) sex	Light microscopy diagnosis	Podocyte infolding	Microsphere	Cluster formation	Microtubule	Dense deposit in GBM	GBM thickening	Immunostaining peripheral granular
Case 1 [2]	31 M	LN class I	Present	Present	Absent	Absent	Absent	Present	All negative
Case 2 [3]	37 F	LN class II	Present	Present	Absent	Present	Absent	Present	G, A, C3, C1q
Case 3 [3]	40 F	LN class II	Present	Present	Absent	Present	Absent	Present	G, A, C3, C1q
Case 4 [4]	30 F	LN class II	Present	Present	Absent	Absent	Present	Present	G, A, C3c, C1q C5b-9
Case 5 [5]	61 F	LN class II	Present	Present	Absent	Absent	Absent	Present	G, M, C1q
Case 6 [6]	29 F	LN class V	Present	Present 50–100 nm	Present	Present	Absent	Present	All negative
Case 7 [6]	46 F	LN class V	Present	Present	Present	Present	Absent	Present	All negative
Case 8 [7]	27 F	LN class V	Present	Present	Present	Present	Present	Present	G, A, M, c3, C1q C5b-9
Case 9 [8]	53 M	LN class V	Present	Present	Absent	Present	Absent	Present	All negative
Case 10 [9]	23 F	LN class V	Present	Present	Present	Present	Present	Present	G
Case 11 [2]	31 F	LN class V	Present	Present	Absent	Present	Present	Present	G
Case 12 [10]	24 F	LN class V	Present	Present	Absent	Present	Present	Present	G, M, C1q
Case 13 [11]	49 M	MPGN type 3	Present	Absent	Absent	Present	Absent	Absent	G, A
Case 14 [2]	20 F	MGA	Present	Present	Absent	Absent	Absent	Present	G+−
Case 15 [2]	47 F	MGA	Present	Present	Absent	Absent	Present	Present	G, A, M
Case 16 [3]	51 F	MGA	Weak	Present	Absent	Present	Absent	Present	All negative
Case 17 [2]	30 F	MGA	Present	Present	Absent	Absent	Absent	Present	G
Case 18 [12]	54 F	FSGS + bubbling	Present	Present	Present	Absent	Absent	Present	All negative
Case 19 [13]	57 F	FSGS + bubbling	Weak	Present 6–310 nm	Absent	Absent	Absent	Present	All negative
Case 20 [14]	45 M	FSGS + bubbling	Weak	Present 50–70 nm	Absent	Present	Absent	Present	G, A, C3
Case 21 [15]	42 F	FSGS + MGN	Weak	Present 65–130 nm	Present	Absent	Absent	Present	G+−
Case 22 [16]	69 F	MGN stage 3 > 2	Present	Present	Present	Absent	Absent	Present	G, A, M, C3
Case 23 [17]	46 M	MGN stage 3 > 2	Present	Present 40–60 nm	Present	Present	Absent	Present	G+−, anti HBVs Ag
Case 24 [18]	59 M	MGN stage 3 > 2	Present	Present	Absent	Absent	Absent	Present	M
Case 25 [19]	45 F	MGN stage 3 > 2	Present	Present 50–150 nm	Present	Present	Absent	Present	G, A, C3

LN lupus nephritis, *MPGN* membranoproliferative glomerulonephritis, *MGA* minor glomerular abnormality, *FSGS* focal segmental glomerulosclerosis, *MGN* membranous glomerulonephritis *G* IgG, *A* IgA, *M* IgM

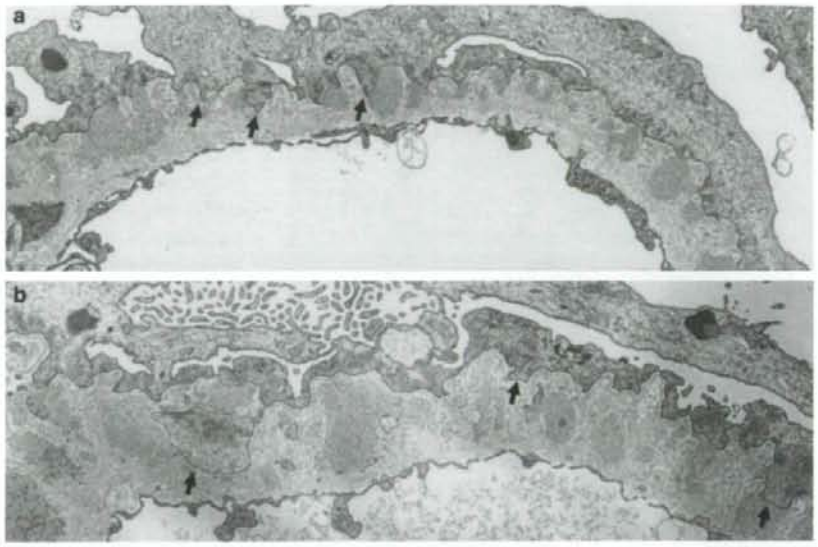


Fig. 9 Electron micrograph of membranous glomerulonephritis stage 2 (**a**) and stage 3 (**b**). Primary podocytic infolding type A (Fig. 7) can be found also in membranous nephropathy stage 2 and stage 3 (*arrows*) (×10,000)

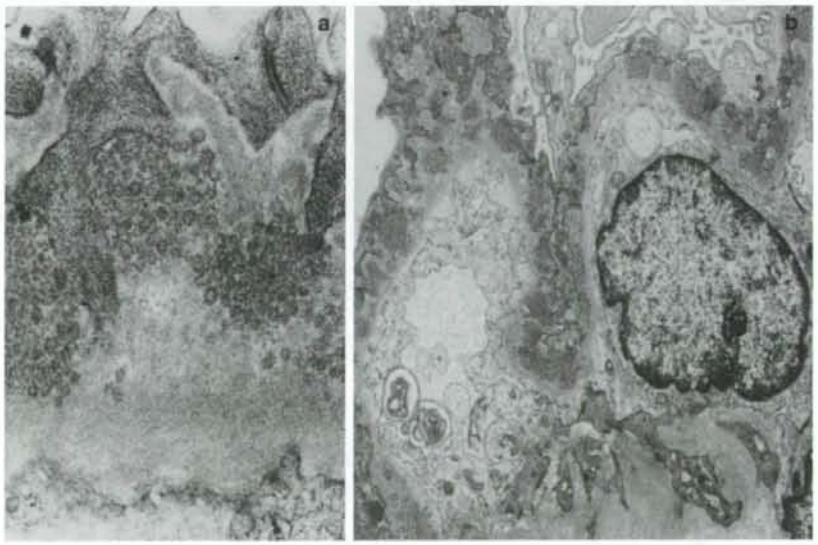


Fig. 10 **a** Electron micrograph of case 22 shows a primary podocytic infolding, around the tip of which clusters of the microspheres measuring 50–70 nm can be seen in the GBM (×30,000). **b** The clustered microspheres distributed mainly from the subepithelial space to the middle layer of the lamina densa in the GBM. A distribution of clustered microspheres is similar to a distribution of electron-dense deposits of membranous glomerulopathy, stage 2 (×3,000)

deposits but only podocytic infolding lesion were divided into a group positive for immunoglobulins or complements or both and a group negative for all immunoglobulins and complement. These findings suggest that some cases of collagen disease-associated PIG are not distinct from a variant of lupus nephritis, class V. In other words, lupus nephritis, class V can makes the transition to PIG. However, how PIG can combine with lupus nephritis in only a very small population is unclear.

In patients with collagen disease-irrelevant PIG, no electron-dense deposits were found in the GBM. However, light-microscopic diagnoses included membranous glomerulonephritis with positive immunoglobulins or complement or both in the glomerular peripheral loops and focal segmental glomerulosclerosis (FSGS) without staining for immunoglobulins. A differentiation of PIG from membranous nephropathy is to be discussed. Primary podocytic infolding (type A in Fig. 7) can also be found in membranous nephropathy stage 2 or stage 3 together with a reaction of the basement membrane surrounding electron-dense deposits (Fig. 9a, b) and in some cases of minimal change nephrotic syndrome [20]. Therefore, primary

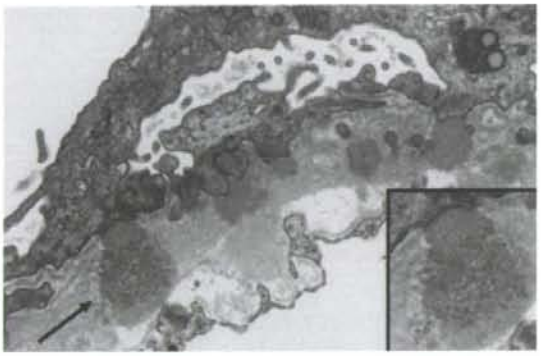


Fig. 11 Primary membranous glomerulonephritis, stage 2 in a 70-year-old man shows microspheres in a limited part of an electron-dense deposit (*arrow*) (×10,000)

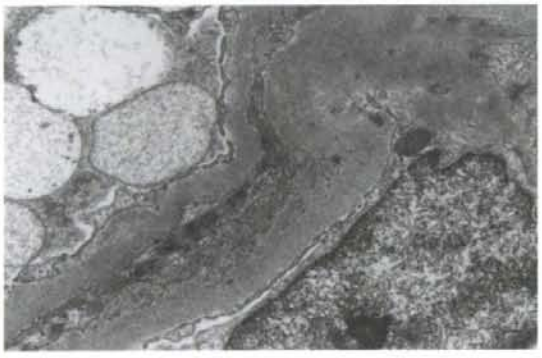


Fig. 12 Electron micrograph of IgA nephropathy in 41-year-old man. Tight aggregation of SMPs beneath podocytes can be distinguished from the PIG of the present study, because the aggregation can usually be found in a specific, limited area, where glomerular capillary tufts come close together with back-to-back contact of the podocytes (×10,000)

podocytic infolding alone cannot be a critical finding for differentiation. Microstructures in the GBM such as microspheres or microtubular structures or both(type B and type C in Fig. 7) are usually not found in membranous nephropathy excepting granular and membranous debris in stage 3 membranous nephropathy [21, 22] and in rare cases of HBV-associated glomerulonephritis [21, 23]. From this point of view, our patient 23 (Fig. 6), who was positive for HBV antigens might have a variant of membranous glomerulonephritis. Fourteen patients with clustered microspheres in the GBM have recently been reported [24]. The morphological characteristics have been described as cluster formation of spherular structures in the subepithelial space with an average diameter of 84.5 nm and electron lucent core associated with a targetoid appearance, which shows morphological similarity to nuclear pore complexes. However, the spherular structure was negatively stained with antinuclear pore antibody and antineutral endopeptidase (NEP) (CD 10) antibody. Membranous nephropathy with microspheres has also been reported in a male infant whose mother had a deficiency of NEP and had become immunized against the antigen before delivery [25]. These two reports considered the glomerulopathy to be a subtype of membranous glomerulonephritis. Our patient 22 showed a primary podocytic infolding, around the tip of which clusters of the microspheres could be seen in the GBM (Fig. 10a). The glomeruli were strongly positive for all immunoglobulins and complement. The distribution of clustered microspheres distributed was mainly from the subepithelial space to the middle layer of the lamina densa in the GBM, similar to that of membranous glomerulopathy, stage 2 (Fig. 10b). Therefore, this case can be also interpreted as a subtype of membranous glomerulonephritis. In fact, there was a case of membranous glomerulonephritis that showed microspheres in limited parts of the electron dense deposits (Fig. 11). On the other hand, microspheres with or without cluster formation, in type B and type C of some cases of PIG showed positive staining with anti-human immunoglobulins or complement or both antibodies. From this point of view, it may be difficult to discriminate immunoglobulin-positive PIG from a subtype of membranous glomerulonephritis. However, we can say that a subtype of membranous glomerulonephritis shows clusters of microspheres mainly in the subepithelial space of the GBM, which is strongly positive for immunoglobulins and complement. Meanwhile, more than half of the cases of the PIG did not show cluster formation and microspheres distributed throughout the GBM, but did not confine in the subepithelial space. Moreover, in patients with collagen disease-irrelevant PIG who did not show electron-dense deposits but show only microspheres in the GBM, glomerular peripheral loops were either negative or positive for immunoglobulins. Likewise in patients with collagen disease-associated PIG without electron dense deposits in the GBM, glomerular capillary loops were either positive or negative for immunoglobulins. Why PIG can be either positive or negative for immunoglobulins is unknown. At least, collagen disease-irrelevant PIG showing FSGS with negative staining for immunoglobulins can be clearly differentiated from a subtype of membranous nephropathy.

Villous cytoplasmic protrusion of endothelial cells into the GBM and of mesangial cells into the mesangial matrix were also found in patients with PIG. Therefore, some intramembranous microstructures can be derived from the protruded cytoplasm of endothelial cells or mesangial cells or both. However, these villous protrusions were found in limited areas but were not a generalized manifestation, whereas the distribution of podocytic infolding was diffuse

and global, and was not limited to a segmental part of the GBM. Therefore, it is conceivable that most microspheres or microtubules or both can be derived from podocytes. These findings support to use the term of podocytic, but not endothelial or mesangial, infolding glomerulopathy.

The pathogenic mechanism of PIG is unknown. Hydronephrosis was found in three of 25 patients. However, no study has shown that experimental hydronephrosis can induce podocytic infolding. Nakajima et al. [26] have used immune electron microscopy to analyze extracellular organized structures from various kinds of glomerular diseases. The organized structures were divided into microspherical structures and thread-like structure. C1s, C3d, and C9 but not IgA, IgG, fibrinogen, C1q, C1r, C3c, C4, or C5 were localized to individual membrane-like structures of both microspherical and thread-like structures. Higlais et al. [27] performed an immunohistochemical study of complement C5b-9 complexes in several human kidney diseases and have shown positive reactions on round extracellular particles and on striated membranous structures in the GBM. Therefore, the mechanism of podocytic infolding might be related to the role of special types of complement activation in situ on the microstructures [27]. Burkholder et al. have reported 55 cases in which extracellular spherical microparticles (SMPs) were found in the GBM among 1,400 renal biopsies [28]. The SMPs usually formed clusters in the extracellular matrix and have a mean size of 50–58 nm. A viral origin of SMPs was suspected in some cases. However, most SMPs were considered to be non-viral, perhaps particles discharged from cells during glomerular injury or spherical lipoprotein crystalline bodies. However, reported SMPs seemed to be heterogeneous and included tight aggregations of SMPs beneath portions of podocytes, subepithelial loose clusters of SMPs with a lucent core, and intramembranous clusters of SMPs. At least, tight aggregations of SMPs beneath podocytes as shown in Fig. 12 can be distinguished from the PIG of the present study because the aggregation can be usually found in limited, specific areas, where glomerular capillary tufts come close together with back-to-back contact of podocytes (Fig. 12). Moreover, the authors did not mention podocytic infolding as an origin of SMP. We emphasize that microspheres or microtubular structures or both of PIG can be derived by budding from the cell membrane of primary podocytic infolding tips, partially by breaking off from disintegrated podocytic cell processes and drifting into the GBM. Thickening of the GBM containing microspheres might be caused by a loss of balance between biosynthesis and degradation of the matrix of the podocytes.

In conclusion, we propose a new disease entity, podocytic infolding glomerulopathy, as a common basis of all 25 patients, because we suspect that microspheres or microtubular structures or both can be derived from primary podocytic infolding.

References

1. Word Health Organization. Section 1. Classification of Glomerular Diseases. Glossary of Terms. In: Churg J, Bernstein J, Glassock RJ, editors. Renal disease classification and atlas of glomerular diseases. 2nd ed. Tokyo: Igaku-Shoin; 1995. p. 3–26.
2. Sato H, Saito T, Yoshinaga K. Intramembranous fine deposit disease associated with collagen disorder: a new morphological entity? Virchows Arch A Pathol Anat Histopathol. 1992;420: 447–51.
3. Sugiyama H, Maruyama M, Morinaga H, Inoue T, Takiue K, Kikumoto Y, et al. Unique microstructures and podocytic infolding in glomerular basement membrane associated with collagen diseases: a report of three cases.Clin Exp Nephrol. 2008, doi:10.1007/s10157-008-0098-6.
4. Sato M, Kogure T, Kanemitsu M. A case of systemic lupus erythematosus showing invagination of the podocyte into the glomerular basement membrane: an electron microscopic observation of a repeated-renal biopsy. Clin Exp Nephrol. 2008, doi:10.1007/s10157-008-0091-0.
5. Kitazawa K, Joh K, Akizawa T. A case of lupus nephritis coexisting with podocytic infolding associated with Takayasu's arteritis. Clin Exp Nephrol. 2008. doi:10.1007/s10157-008-0094-x.
6. Jinguuji Y, Nukui I, Wakasugi M, Yamashita H. Two cases of systemic lupus erythematosus complicated by hydronephrosis and unique small structures observed in the glomerular basement membrane. Clin Exp Nephrol. 2008. doi:10.1007/s10157-008-0092-z.
7. Fujigaki Y, Muranaka Y, Sakakima M, Ohta I, Sakao Y, Fujikura T, et al. Analysis of intra-GBM microstructures in a SLE case with glomerulopathy associated with podocytic infolding. Clin Exp Nephrol. 2008. doi:10.1007/s10157-008-0095-9.
8. Miura N, Mori Y, Yoshino M, Suga N, Kitagawa W, Yamada H, et al. Membranous nephropathy (bubbling appearance and spike formation) without immunoglobulin deposition in a patient with systemic lupus erythematosus. Clin Exp Nephrol. 2008. doi: 10.1007/s10157-008-0096-8.
9. Mii A, Shimizu A, Masuda Y, Ishizaki M, Sato S, Hara K, et al. A case of lupus nephritis with diffuse podocytic infolding 5 into the glomerular basement membrane. Clin Exp Nephrol. 2008. doi: 10.1007/s10157-008-0097-7.
10. Tanaka M, Watanabe K, Asahi K, Katoh T, Watanabe T. Lupus nephritis with podocytic infolding and intramembranous microstructures. Clin Exp Nephrol. 2008. doi:10.1007/s10157-008-0090-1.
11. Koike K, Utsunomiya Y, Ito Y, Tokudome S, Miyazaki Y, Suzuki T, et al. A case of glomerulopathy showing podocytic infolding in association with Sjögren's syndrome and primary biliary cirrhosis. Clin Exp Nephrol. 2008. doi:10.1007/s10157-008-0093-y.
12. Matsuo T, Kobayashi Y, Nemoto N, Sano T, Kamata K, Shigematsu H. A nephrotic case of vesicoureteral reflux representing focal segmental glomerulosclerosis associated with podocytic infolding lesions. Clin Exp Nephrol. 2008. doi:10.1007/s10157-008-0086-x.
13. Arifa N, Nakashima Y, Yan Z, Le VT, Taguchi T, Nishioka K, et al. Unusual glomerulopathy with atypical thickening of glomerular basement membrane and intramembranous microparticles. Clin Exp Nephrol. 2008. doi:10.1007/s10157-008-0103-0.

14. Inoue Y, Kanda Y, Kinoshita C, Kanda C. Intramembranous microspherical structures in focal segmental glomerulosclerosis:a case report. Clin Exp Nephrol. 2008. doi:10.1007/s10157-008-0101-2.
15. Yamada S, Masutani K, Katafuchi R, Fujigaki Y, Muranaka Y, Tsuruya K, et al. Focal segmental glomerulosclerosis with intramembranous vesicle-like microstructures and podocytic infolding lesion. Clin Exp Nephrol. 2008. doi:10.1007/s10157-008-0088-8.
16. Nomura S, Inoue-Kiyohara M, Oosugi K, Ishikawa E, Obe T, Horiki T, et al. Unusual glomerulopathy with aggregated subepithelial microspheric particles resembling membranous nephropathy: a variant of glomerulopathy associated with podocytic infolding? Clin Exp Nephrol. 2008. doi:10.1007/s10157-008-0089-7.
17. Nishi S, Imai N, Saito T, Ueno M, Arakawa M, Oota T, et al. A nephropathy presenting the microparticles in the glomerular basement membrane in a patient of hepatitis B viral infection. Clin Exp Nephrol. 2008. doi:10.1007/s10157-008-0087-9.
18. Yoshimura K, Joh K, Kitamura H, Takahashi Y, Yokote S, Kasai K, et al. A case report of glomerulopathy-associated podocytic infolding 5 in a patient with tumor lysis syndrome. Clin Exp Nephrol. 2008. doi:10.1007/s10157-008-0099-5.
19. Masuda Y, Mii A, Shimizu A, Fujita E, Aki K, Ishikawa K, et al. Invagination and infolding of podocytes in glomerular basement membrane in the cases of primary membranous nephropathy. Clin Exp Nephrol. 2008. doi:10.1007/s10157-008-0100-3.
20. Fujigaki Y, Kobayashi S, Nagase M, Honda N, Otawara Y, Tajima A, et al. Indentation of the glomerular basement membrane in cases with minor abnormalities. J Clin Microscopy. 1987;20:5–6.
21. Schwartz MM. Membranous glomerulonephritis. In: Jeannette JC, Olson JL, Schwartz MM, Silva FG, editors. Heptinstall's Pathology of the Kidney. 6th ed. Philadelphia: Lippincott/Williams &Wilkins; 2007. p. 205–51.
22. D'Agati, Jennette JC, Silva FG. 7. Membranous glomerulopathy. In: King DW, editor. Atlas of Nontumor Pathology. 1st Series, Fascicle 4. Non-Neoplastic Kidney Diseases. American Registry of pathology, Armed Forces Institute of Pathology. Silver Spring: ARP Press; 2005. pp 161–188.
23. Venkataseshan VS, Lieberman K, Kim DU, Thung SN, Dikman S, D'Agati V, et al. Hepatitis-B-associated glomerulonephritis: pathology, pathogenesis, and clinical course. Medicine (Baltimore). 1990;69:200–16.
24. Kowalewska J, Smith KD, Hudkins KL, Chang A, Fogo AB, Houghton D, et al. Membranous glomerulopathy with spherules: an uncommon variant with obscure pathogenesis. Am J Kidney Dis. 2006;47:983–92.
25. Debiec H, Guigonis V, Mougenot B, Haymann J-P, Bensman A, Deschenes G, et al. Antenatal membranous glomerulonephritis with vascular injury induced by anti-neutral endopeptidase antibodies: toward new concepts in the pathogenesis of glomerular diseases. Am Soc Nephrol. 2003;14:27–32.
26. Nakajima M, Hewitson TD, Mathews DC, Kincaid-Smith P. Localization of complement components in association with glomerular extracellular particles in various renal diseases. Virchows Arch A Pathol Anat. 1991;419:267–72.
27. Hinglais N, Kazatchkine ND, Bhakdi S, Appay M-D, Mandet C, Grossetete J, et al. Immunohistochemical study of the C5d-9 complex of complement in human kidneys. Kidney Int. 1986;30:399–410.
28. Burkholder PM, Hyman LR, Barber TA. Extracellular cluster of spherical microparticle in glomeruli in human renal glomerular disease. Lab Invest. 1973;28:415–25.

Nephrol Dial Transplant (2008) 23: 3915–3920
doi: 10.1093/ndt/gfn394
Advance Access publication 20 July 2008

Original Article

NDT
Nephrology Dialysis Transplantation

The improvement of renal survival with steroid pulse therapy in IgA nephropathy

Ritsuko Katafuchi[1], Toshiharu Ninomiya[2], Tohru Mizumasa[1], Kiyoshi Ikeda[1], Harumitsu Kumagai[1], Masaharu Nagata[2] and Hideki Hirakata[1]

[1]The Kidney Unit, Fukuoka Red Cross Hospital and [2]The Department of Medicine and Clinical Science, Graduate School of Medical Science, Kyushu University, Fukuoka, Japan

Abstract

Background. The benefits of steroid therapy in immunoglobulin A nephropathy (IgAN) have not been established.

Methods. The effect of steroids on kidney survival was retrospectively investigated in 702 patients with IgAN by multivariate analyses.

Results. There were 295 men and 407 women. The median follow-up period was 62 months. One hundred and ninety-four patients were treated with oral steroids (oral steroid group). Thirty-four patients were treated with methylprednisolone (mPSL) pulse therapy (pulse steroid group) followed by oral prednisolone (PSL). In 474 patients, no steroid was used (no steroid group). The urinary protein-creatinine ratio and histological grade were significantly different among treatment groups and were highest in the pulse steroid group followed by the oral steroid group and lowest in the no steroid patients. Serum creatinine was significantly higher in the pulse steroid group than in other two groups. Eighty-five patients developed end-stage renal failure (ESRF) requiring haemodialysis. In multivariate analysis, steroid pulse therapy significantly decreased the risk of ESRF while oral steroid treatment did not improve renal survival in this cohort.

Conclusion. We found that pulse steroid therapy improved kidney survivals in IgAN. Since the clinical findings and histological grade were the most severe in patients treated with mPSL pulse therapy, such therapy may prevent progression of IgAN.

Keywords: histological grade; IgA nephropathy; multivariate analysis; steroid pulse therapy; the Cox proportional hazards model

Introduction

Since Kobayashi *et al.* first reported the efficacy of steroid treatment in patients with immunoglobulin A nephropathy (IgAN) in 1986 [1], there has been widespread interest in corticosteroid therapy for IgAN [2–17]. However, the efficacy of steroid treatment has not yet been clarified.

In 2003, we reported a randomized control trial (RCT) in patients with IgAN with a glomerular score between 4 and 7, and showed that a low-dose prednisolone (PSL) protocol (20 mg/day as initial dose) had an anti-proteinuric effect but no effect on kidney survival [17,18]. We speculated that an insufficient dose of PSL caused a discrepant effect on proteinuria and on kidney survival. Furthermore, pulse therapy has shown promising results in previous trials [14,17].

The aim of this study was to evaluate the effect of steroid treatment on kidney survival in patients with IgAN. We retrospectively investigated the influence of pulse and oral steroid treatment on kidney survival in 702 patients with IgAN by multivariate analyses using the Cox proportional hazards model.

Materials and methods

Study population

Seven hundred and ninety-four patients with primary IgAN, who had been biopsied between October 1979 and September 2002, were followed up at least 1 year later at Fukuoka Red Cross Hospital. Ninety-two biopsies, which contained <10 glomeruli, were excluded. The remaining 702 patients were included in the study. There were no statistical significant differences in age, sex, urine protein-creatinine ratio (UP-UCR), serum creatinine and incidence of end-stage renal failure (ESRF) between the 702 included patients and 92 excluded patient.

Histological grade based on the glomerular score

As previously reported [18,19], the glomerular score was calculated as the sum of indices of the following three

Correspondence and offprint requests to: Ritsuko Katafuchi, National Fukuoka-Higashi Medical Center, 1-1-1, Chidori, Koga city, Fukuoka, 811-3195, Japan. Tel: +81-92-943-2331; Fax: +81-92-943-8775; E-mail: katafuchir@fukuokae2.hosp.go.jp

glomerular lesions: (1) hypercellularity; (2) segmental lesions, including crescent, tuft necrosis, tuft adhesion and segmental sclerosis, and (3) global sclerosis.

The glomerular hypercellularity was defined as three or more nuclei in the mesangial area, or endocapillary hypercellularity in any extent. Each glomerulus in a biopsy specimen was given a point according to the semi-quantitatively evaluated extent of involved area by hypercellularity as follows: 1, no hypercellularity; 2, <25% of the glomerular area; 3, 25–50% and 4, ≥50% of the glomerular area. An average of points in each biopsy was calculated and omitted for the figures below the first decimal place. We termed it as the glomerular hypercellularity index.

Crescents, tuft necrosis, tuft adhesions to Bowman's capsule and segmental sclerosis of glomeruli were evaluated together and termed glomerular segmental lesions, because these lesions were observed to be frequently associated with each other.

The index of segmental lesions and the index of global sclerosis were determined according to the percentage of glomeruli showing each lesion out of the total number of glomeruli in a biopsy sample as follows: 0, none; 1, <10% of glomeruli; 2, 10–25%; 3, 25–50% and 4, ≥50% of glomeruli.

The points of indices of three lesions, i.e. hypercellularity, segmental lesions and global sclerosis, were added together and termed as the glomerular score, ranging from 1 to 12.

Histological grade was determined on the basis of the glomerular score as follows: grade I, glomerular score 1 or 2; grade II, glomerular score 3 or 4; grade III, glomerular score 5 or 6; grade IV, glomerular score 7 or 8 and grade V, glomerular score ≥9.

The glomerular score of all kidney biopsies in the present study were evaluated by one investigator (R.K.). As the glomerular score was determined in 1991, kidney biopsies performed before 1991 were re-evaluated by the same person.

Study design and statistics

The influence of clinical parameters, histological grade and treatment on kidney survival was retrospectively examined. The end point of kidney survival was estimated by ESRF requiring haemodialysis therapy. Clinical parameters used for analyses included age, sex, UP-UCR, blood pressure, serum albumin, serum creatinine, total serum cholesterol, serum triglyceride and serum uric acid. As for the treatment, the effect of the method of steroid therapy, the use of the angiotensin converting enzyme inhibitor (ACE-I) or angiotensin II receptor blocker (ARB), or tonsillectomy was examined. The use of ACE-I or ARB was defined as treatment for at least 6 months. Any antihypertensive agent was permitted to control blood pressure during the follow-up.

The SAS software package was used to perform all statistical analyses. Renal survival in all patients was assessed by the life table method and the log rank statistic. Comparisons among the groups were assessed by the chi-square method, analysis of variance or Kruskal–Wallis method. The statistical significances of the differences in mean of continuous variables, median values or frequencies of categorical variables between the two groups were determined by the multiple *t*-test, Mann–Whitney *U*-test or chi-square test with Bonferroni correction.

The crude or multivariate-adjusted hazard ratios (HRs) and 95% confidence intervals (CIs) were estimated with the use of the Cox proportional hazards model. In the multivariate model, we selected clinically or biologically plausible risk factors for the determination of kidney survivals as baseline potential confounding factors [19,20], and used the backward procedure with $P < 0.05$ required to retain each variable in the model including age and sex. Because 21 patients changed treatment groups during the follow-up (12 patients, no steroid to oral steroid; 5 patients, no steroid to pulse therapy; 4 patients, oral steroid to steroid pulse therapy), we also estimated the effect of steroid therapy using the time-dependent Cox proportional hazard regression model including the time-dependent status of treatment and covariates.

$P < 0.05$ was considered statistically significant.

Table 1. Baseline characteristics of study population

Variables	
Number	702
Duration of follow-up (months)	62 (6–281)
Age (years)	33 ± 14
Men/women	295/407
Systolic blood pressure (mmHg)	126 ± 19
Diastolic blood pressure (mmHg)	75 ± 14
Urinary protein-creatinine ratio	1.5 ± 1.9
Urinary haematuria	2.2 ± 0.9
Serum albumin (g/dl)	4.2 ± 0.5
Serum creatinine (mg/dl)	0.98 ± 0.58
Serum uric acid (mg/dl)	5.6 ± 1.6
Serum total cholesterol (mg/dl)	198 ± 45
Serum triglycerides (mg/dl)	123 ± 95
Serum IgA (mg/dl)	366 ± 126
Histological grade, *n* (%)	
Grade I (glomerular score 1 or 2)	114 (16.2)
Grade II (glomerular score 3 or 4)	208 (29.6)
Grade III (glomerular score 5 or 6)	203 (28.9)
Grade IV (glomerular score 7 or 8)	145 (20.7)
Grade V (glomerular score >9)	32 (4.6)
Steroid therapy, *n* (%)	228 (32.3)
Use of ACE-I or ARB, *n* (%)	241/659 (36.6)
Tonsillectomy, *n* (%)	28/623 (4.5)

ACE-I, angiotensin converting enzyme inhibitor; ARB, angiotensin-II receptor blocker.
Duration of follow-up is median (range).
Values are means ± standard deviation or frequencies.
Note: To convert serum creatinine in mg/dl to μmol/l, multiply by 88.4. To convert serum uric acid in mg/dl to μmol/l, multiply by 59.5. To convert triglyceride in mg/dl to mmol/l, multiply by 0.0113. To convert cholesterol in mg/dl to mmol/l, multiply by 0.026. To convert serum IgA in mg/dl to g/l, multiply by 0.01.

Results

The baseline characteristics of the study population

The baseline characteristics of the patients are shown in Table 1. The median follow-up period was 62 months, ranging from 6 to 281 months. Eight patients developed ESRF

Table 2. Baseline characteristics of treatment groups

Variables		No steroid	Oral steroid	Pulse steroid	*P*
Number		474	194	34	
Number of ESRF		57	21	7	
Median duration of follow-up, months (range)		62 (6–281)	69 (7–240)	44 (11–147)[b]	0.031
Age (years)		33 ± 14	33 ± 13	33 ± 18	n.s.
Men/women		192/282	84/110	19/15	n.s.
Systolic blood pressure (mmHg)		125 ± 19	127 ± 19	132 ± 24	n.s.
Diastolic blood pressure (mmHg)		74 ± 14	77 ± 15[a]	79 ± 18	0.012
Urinary protein-creatinine ratio		1.1 ± 1.6	2.1 ± 1.9[a]	3.9 ± 3.2[a,b]	<0.0001
Urinary haematuria		2.1 ± 1.0	2.4 ± 0.9[a]	2.8 ± 0.5[a]	<0.0001
Serum albumin (g/dl)		4.3 ± 0.5	4.1 ± 0.6[a]	3.7 ± 0.6[a,b]	<0.0001
Serum creatinine (mg/dl)		0.9 ± 0.6	1.0 ± 0.4	1.5 ± 1.0[a,b]	<0.0001
Serum uric acid (mg/dl)		5.5 ± 1.6	5.7 ± 1.6	6.3 ± 1.5[a]	0.016
Serum total cholesterol (mg/dl)		193 ± 43	205 ± 43[a]	238 ± 54[a,b]	<0.0001
Serum triglycerides (mg/dl)		120 ± 100	118 ± 68	190 ± 118[a,b]	0.0001
Serum IgA (mg/dl)		362 ± 121	385 ± 135[a]	313 ± 116[b]	0.004
Histological grade, *n* (%)				[a,b]	<0.0001
Grade I (glomerular score 1 or 2)	*n* (%)	112 (23.6)	1 (0.5)	1 (2.9)	
Grade II (glomerular score 3 or 4)	*n* (%)	169 (35.7)	37 (19.1)	2 (5.9)	
Grade III (glomerular score 5 or 6)	*n* (%)	112 (23.6)	82 (42.3)	9 (26.5)	
Grade IV (glomerular score 7 or 8)	*n* (%)	71 (15.0)	60 (30.9)	14 (41.2)	
Grade V (glomerular score ≥9)	*n* (%)	10 (2.1)	14 (7.2)	8 (23.5)	
Use of ACE-I or ARB, number/total		133/441	90/187[a]	18/32[a]	<0.0001
Tonsillectomy, number/total		15/418	7/173	6/32[a,b]	0.003

ESRF, end-stage renal failure; n.s, not significant difference; no steroid, no steroid therapy; oral steroid, oral prednisolone therapy; pulse, 1000 mg of methyl-prednisolone pulse therapy followed by oral prednisolone; ACE-I, angiotensin converting enzyme inhibitor; ARB, angiotensin-II receptor blocker.

Duration of follow-up is median (range).

Values are frequencies or means ± standard deviation or frequencies.

[a]Significantly different compared to the no steroid group.

[b]Significantly different compared to the oral steroid group.

Note: To convert serum creatinine in mg/dl to μmol/l, multiply by 88.4. To convert serum uric acid in mg/dl to μmol/l, multiply by 59.5. To convert triglyceride in mg/dl to mmol/l, multiply by 0.0113. To convert cholesterol in mg/dl to mmol/l, multiply by 0.026. To convert serum IgA in mg/dl to g/l, multiply by 0.01.

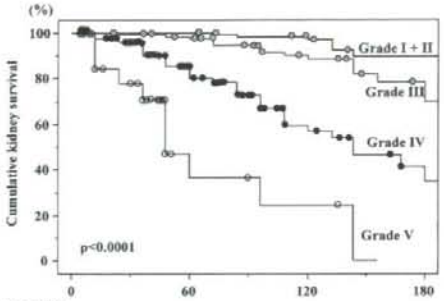
within 1 year of the follow-up. The mean age at biopsy was 33 ± 14 years. There were 295 men and 407 women. The mean systolic or diastolic blood pressure (DBP) was 126 ± 19 mmHg and 75 ± 14 mmHg, respectively. The mean UP-UCR was 1.5 ± 1.9, and the mean degree of haematuria was 2.2 ± 0.9. The mean serum creatinine level was 0.98 ± 0.58 mg/dl (87 ± 51 μmol/l). The mean serum IgA level was 366 ± 126 mg/dl (3.66 ± 1.26 g/l).

One hundred fourteen patients were classified in histological grade I, 208 in grade II, 203 in grade III, 145 in grade IV and 32 in grade V.

Out of 702 patients, 228 patients were treated with steroids. Two hundred forty-one were treated with ACE-I or ARB. Of these, 167 were hypertensive and the remaining 74 patients were normotensive. During the follow-up, 28 patients had tonsillectomy.

The baseline characteristics in each treatment groups

Methylprednisolone (mPSL) pulse therapy, 1 g/day for three consecutive days followed by 30 mg of PSL, was undertaken in 34 patients (pulse steroid group). PSL was tapered and maintained for at least 2 years. Oral steroid therapy was given to 194 patients (oral steroid group). In 46 patients, initial dose of PSL was 60 mg for 4 weeks and PSL was tapered off within 1 year. In 148 patients, the initial dose of PSL was 20 mg or 30 mg for 1 month. PSL was tapered and maintained for at least 2 years. The remaining 474 patients were treated with an anti-platelet agent or with no medication (no steroid group). Table 2 shows the baseline characteristics in each treatment group. UP-UCR was significantly different among groups and was 1.1 ± 1.6, 2.1 ± 1.9 and 3.9 ± 3.2 in no steroid, oral steroid and pulse steroid groups, respectively. UP-UCR was significantly higher in both oral and pulse steroid group than in the no steroid group ($P < 0.0001$). Furthermore, UP-UCR was significantly higher in the pulse steroid group than in the oral steroid group ($P < 0.0001$). Serum creatinine was 0.9 ± 0.6, 1.0 ± 0.4 and 1.5 ± 1.0 in the no steroid, oral steroid and pulse groups, respectively, and was significantly higher in the pulse steroid patients compared to the other two groups. The severity of histological grade was significantly different among treatment groups. In the no steroid group, the percentage of patients with histological grade I–V was 23.6, 35.7, 23.6, 15.0 and 2.1, respectively. In the oral steroid group, the percentage of patients with histological grade I–V was 0.5, 19.1, 42.3, 30.9 and 7.2, respectively. The percentage of patients with histological grade I–V was 2.9, 5.9, 26.5, 41.2 and 23.5, respectively, in the pulse steroid group. The histological grade was highest in the pulse steroid group followed by the oral steroid group and lowest in the no steroid patients.



Patients at risk

Grade I+II	322	162	51	13
Grade III	203	127	43	6
Grade IV	145	76	21	5
Grade V	32	5	1	0

Fig. 1. Kidney survival curve in each histological grade. The patients with histological grades I and II were analysed together due to the small number of end-stage renal failure incidence. Kidney survivals significantly differed among the histological grades ($P < 0.0001$). The numbers of patients remaining at 60, 120 and 180 months of follow-up in each histological grade are shown at the bottom.

Cumulative kidney survival in all patients

During the follow-up period, 85 patients developed ESRF. The cumulative kidney survival rate was 92.1% at 5 years and 81.6% at 10 years from biopsy.

Kidney survival rate according to histological grade analysed by the life table method

The kidney survival curve analysed by the life table method in each histological grade is shown in Figure 1. The patients with histological grades I and II were analysed together due to the small number of ESRF incidence. Five-year kidney survival in grade I+II, III, IV and V was 99.3, 97.5, 80.5 and 36.7%, respectively. Ten-year kidney survival in grade I+II, III, IV and V was 95.6, 88.3, 57.2 and 24.5%, respectively. Kidney survivals differed significantly among the histological grades ($P < 0.0001$).

Risk factors for the development of ESRF

Crude or multivariate-HRs for the development of ESRF are shown in Table 3. In crude analysis, age, UP-UCR, serum creatinine, histological grade, systolic blood pressure (SBP), serum total cholesterol, serum triglyceride and serum uric acid significantly increased the risk of developing ESRF. HR of ESRF for women was significantly lower than men. Higher serum albumin was associated with a significant risk reduction of ESRF.

In multivariate analysis, UP-UCR, serum creatinine, serum triglycerides and serum uric acid significantly increased the risk of the development of ESRF ($P < 0.05$, $P < 0.01$, $P < 0.05$, $P < 0.05$, respectively). Serum albumin significantly decreased the risk of ESRF ($P < 0.01$). Multivariate adjusted HRs for the development of ESRF in histological grades III, IV and V were 3.56, 8.64 and 5.74, respectively, and significantly increased compared to grade I+II ($P < 0.05$, $P < 0.01$, $P < 0.05$, respectively). As for the steroid treatment, HR of steroid pulse therapy decreased significantly compared to the no steroid group (HR 0.14, 95% CI 0.05–0.44, $P < 0.01$). On the other hand, HR for the development of ESRF of the oral steroid group did not show any significant difference compared to the no steroid group (HR 0.61, 95% CI 0.30–1.22). These findings were still observed even in the analysis using the time-dependent Cox hazard model with the status of steroid treatment during the follow-up (oral steroid, HR 0.58, 95% CI 0.29–1.17; pulse therapy, HR 0.15, 95% CI 0.05–0.43, $P < 0.01$). The use of ACE-I or ARB significantly decreased the risk of renal death ($P < 0.01$). Age, sex, serum total cholesterol, tonsillectomy and SBP did not show any association with ESRF in multivariate analysis. We selected SBP rather than DBP in the multivariate analysis, because the likelihood ratio of the model for SBP showed better fitting than that for DBP.

Adverse effect

In the oral steroid group, two patients developed steroid-induced psychosis and were treated well by the psychiatrist. One patient refused to take PSL after 8 months because of palpitation, increased perspiration and facial blushing. Three patients complained of insomnia and palpitation but were able to tolerate the protocol. One patient had aseptic necrosis of medial epicondyle of left femoral bone after two courses of the low-dose protocol of PSL treatment. The duration between the beginning of PSL and the onset of left knee pain was 6 years. Another patient developed diabetes mellitus, and PSL was tapered immediately. One patient suffered from herpes zoster after 4-month steroid therapy and was treated with aciclovir. In the mPSL pulse group, one patient developed tuberculosis of left cervical lymph node 4 months after the beginning of mPSL and was successfully treated with anti-tubercular agents. The other two patients developed herpes zoster 4 or 5 months after steroid therapy and were treated with acyclovir.

Discussion

A variety of studies have been investigated to confirm the effect of steroid treatment on amelioration of the clinical course of IgAN [1–17]. However, the efficacy of steroid therapy in patients with IgAN has not yet been established.

In our hospital, the treatment of IgAN patients has changed over time. Since the initial 1986 publication of the effects of steroids in IgAN by Kobayashi *et al.* [1], we started a pilot study of steroid treatment with 60 mg of PSL as initial dose, gradually tapered off within 1 year. We realized the necessity for a RCT to assess the efficacy of steroid treatment in IgAN and performed between 1991 and 1995 a RCT in IgAN with a glomerular score ranging from 4 to 7. A low-dose PSL protocol (initial dose of PSL; 20 mg/day) showed only an anti-proteinuric effect and no effect on kidney survival [18]. Moreover, the protocol failed to demonstrate an anti-proteinuric effect in patients with massive proteinuria, i.e. UP-UCR exceeding 3. Since the amount of proteinuria has been reported to be one of the most important prognosticators in IgAN [21–25] and the effect of steroid therapy on preventing the progression

Table 3. Crude or multivariate-adjusted hazard ratios for the development of end-stage renal failure

		Univariate analysis		Multivariate analysis (backward method)	
Risk factor	Scale	HR	95% CI	HR	95% CI
Age (years)	(every 10 years)	1.43	(1.23–1.66)**	1.03	(0.81–1.31)
Women	(versus men)	0.52	(0.34–0.80)**	1.29	(0.67–2.47)
UP-UCR	(every 1)	1.29	(1.22–1.35)**	1.16	(1.02–1.31)*
Serum creatinine	(every 1 mg/dl)	3.91	(3.26–4.69)**	3.95	(2.50–6.25)**
SBP	(every 10 mmHg)	1.34	(1.21–1.48)**	1.03	(0.87–1.20)
Serum albumin	(every 1 g/dl)	0.27	(0.19–0.37)**	0.49	(0.29–0.84)**
Serum total cholesterol	(every 10 mg/dl)	1.01	(1.04–1.13)**	–	–
Serum triglycerides	(every 10 mg/dl)	1.00	(1.03–1.06)**	1.03	(1.00–1.06)*
Serum uric acid	(every 1 mg/dl)	1.57	(1.40–1.75)**	1.3	(1.04–1.63)*
Histological grade					
Grade I+II (glomerular score 1–4)		1 (reference)		1 (reference)	
Grade III (glomerular score 5 or 6)		3.58	(1.40–9.14)**	3.56	(1.11–11.39)*
Grade IV (glomerular score 7 or 8)		16.5	(7.04–38.79)**	8.64	(2.66–28.05)**
Grade V (glomerular score ≥9)		73.9	(29.07–187.78)**	5.74	(1.31–25.08)*
Steroid therapy					
No steroid		1 (reference)		1 (reference)	
Oral steroid		0.88	(0.54–1.46)	0.61	(0.30–1.22)
Pulse steroid		2.6	(1.18–5.71)*	0.14	(0.05–0.44)**
Use of ACE-I or ARB	(versus no use)	1.09	(0.66–1.81)	0.39	(0.21–0.71)**
Tonsillectomy	(versus no tonsillectomy)	0.86	(0.27–2.74)	–	–

UP-UCR, urinary protein-creatinine ratio; ACE-I, angiotensin converting enzyme inhibitor; ARB, angiotensin-II receptor blocker; SBP, systolic blood pressure; HR, hazard ratio.
*$P < 0.05$, **$P < 0.01$.

of IgAN is believed to link closely to reduction in urinary protein [14,26], we speculated that a low-dose PSL protocol was insufficient for the treatment of IgAN with moderate histological severity. Steroid pulse therapy has been done in such patients as those with a glomerular score ≥8, UP-UCR exceeding 3.5 g/day and/or elevated serum creatinine level.

Although the present study is retrospective and it is the limitation of this study, we do believe that it is worthy to report our experience of steroid treatment in a large number of Japanese patients with IgAN in a single centre. We used a multivariate analysis using the Cox proportional hazard model.

In the present study, we found that the influence of steroid therapy on the risk of ESRF was differed between the oral and pulse steroid therapy. The HR for the development of ESRF in the patients with steroid pulse therapy decreased significantly compared to the no steroid group. On the other hand, the impact of oral steroid on the risk of ESRF did not reach statistical significance.

Yoshimura *et al.* first reported the efficacy of mPSL pulse therapy on preventing the progression in eight patients with IgAN showing crescent 10% or more of glomeruli [6]. Two or three courses of 1 g/day mPSL for three consecutive days followed by oral PSL 20 mg/day tapered to 5–10 mg was administrated. Urinary protein excretion significantly decreased, and creatinine clearance significantly increased. After the pulse therapy, they performed second biopsies and found a complete loss or marked decrease in cellular crescent. They suggested that mPSL pulse therapy prevents the progression of IgAN through suppression of new crescent formation as well as transformation of cellular crescent to fibrocellular or fibrous crescent. Hotta *et al.* [11] also reported that not oral but pulse steroid therapy showed a significant effect on remission of proteinuria and haematuria in their retrospective study using multivariate analysis. They also found that clinical remission was closely related to stable kidney function. Their findings are compatible with our results. In 1999, Pozzi *et al.* reported a multicentre, randomized and controlled trial designed to compare the effects of a 6-month steroid course with those of supportive therapy in 86 patients with IgAN [14]. Their steroid regimen was three courses of intravenous mPSL, 1 g/day for three consecutive days plus oral PSL, 0.5 mg/kg, on alternate days for 6 months. After 5 years of follow-up, the risk of a doubling in plasma creatinine levels was significantly lower in the treated patients. Recently, they reported a long-term outcome of their previous control trial [17]. Ten-year renal survival was significantly better in the steroid than in the control group (97% versus 53%, $P = 0.0003$). The end-point of renal survival was a doubling in baseline plasma creatinine levels. The protective effect of mPSL pulse therapy against the progression of IgAN was demonstrated as level-one evidence by their control trial. In the present study multivariate analysis showed that steroid pulse therapy had a significant effect on lowering the risk for the development of ESRF. Considering that the patients treated with steroid pulse therapy were clinically and histologically the most severe cases, steroid pulse therapy seems to be the most effective method among various steroid protocols. However, in our study, the number of patients who received mPSL pulse therapy was only 34. A RCT, which compare the effectiveness of oral steroid and that of pulse steroid therapy, is necessary to reach a conclusion. So far such studies have not yet been reported.

As for the adverse effects of steroid treatment, we got an important message from the patients. Irrespective of the method of steroid therapy, infections such as herpes zoster

and lymph node tuberculosis occurred 4–5 months after the initiation of steroid treatment. We should have paid more attention to prevent such infection during steroid therapy. One patient with low-dose oral steroid treatment developed aseptic necrosis of femoral bone after 6 years of steroid therapy. Long-term steroid therapy should be avoided even with low-dose protocol.

We reported previously that the glomerular score related significantly to the outcome of 248 patients with IgAN in univariate life table analysis [19]. In the present study, we reconfirmed the usefulness of the glomerular score to predict the prognosis in 702 patients with IgAN by multivariate analysis. We have applied steroid pulse therapy in IgAN with histological grade over III, that is, the glomerular score ≥ 5.

In conclusion, we found that pulse steroid therapy improved kidney survival in IgAN. Since the clinical findings and histological grade were the most severe in patients treated with mPSL pulse therapy, such therapy may prevent progression of IgAN.

Acknowledgements. The authors thank Mrs Jean Kawabe for correcting the English.

Conflict of interest statement. None declared.

References

1. Kobayashi Y, Fujii K, Hiki Y *et al.* Steroid therapy in IgA nephropathy: a prospective pilot study in moderate proteinuric cases. *Q J Med* 1986; 61: 935–943
2. Kobayashi Y, Fujii K, Hiki Y *et al.* Steroid therapy in IgA nephropathy: a retrospective study in heavy proteinuric cases. *Nephron* 1988; 48: 12–17
3. Kobayashi Y, Hiki Y, Fujii K *et al.* Moderately proteinuric IgA nephropathy: prognostic prediction of individual clinical courses and steroid therapy in progressive cases. *Nephron* 1989; 53: 250–256
4. Waldo FB, Alexander R, Wyatt RJ *et al.* Alternate-day prednisone therapy in children with IgA-associated nephritis. *Am J Kidney Dis* 1989; 13: 55–60
5. Andreoli SP, Bergstein JM. Treatment of severe IgA nephropathy in children. *Pediatr Nephrol* 1989; 3: 248–253
6. Yoshimura M, Kida H, Saito Y *et al.* Effects of methylprednisolone pulse therapy on progressive IgA nephropathy. *Jap J Nephrol* 1991; 33: 761–768
7. Kobayashi Y, Hiki Y, Kokubo T *et al.* Steroid therapy during the early stage of progressive IgA nephropathy. A 10-year follow-up study. *Nephron* 1996; 72: 237–242
8. Waldo FB, Wyatt RJ, Kelly DR *et al.* Treatment of IgA nephropathy in children: efficacy of alternate-day oral prednisone. *Pediatr Nephrol* 1993; 7: 529–532
9. Tanaka H, Waga S, Kakizaki Y *et al.* Efficacy of long-term alternate day prednisolone therapy in childhood IgA nephropathy. *Clin Exp Nephrol* 1998; 2: 132–136
10. Tsuruya K, Harada A, Hirakata H *et al.* Combination therapy using prednisolone and cyclophosphamide slows the progression of moderately advanced IgA nephropathy. *Clin Nephrol* 2000; 53: 1–9
11. Hotta O, Miyazaki M, Furuta T *et al.* Tonsillectomy and steroid pulse therapy significantly impact on clinical remission in patients with IgA nephropathy. *Am J Kidney Dis* 2001; 38: 736–743
12. Welch TR, Fryer C, Shely E *et al.* Double-blind, controlled trial of short-term prednisone therapy in immunoglobulin A glomerulonephritis. *J Pediatr* 1992; 121: 474–477
13. Yoshikawa N, Ito H, Sakai T, The Japanese Pediatric IgA Nephropathy Treatment Study Group. A controlled trial of combined therapy for newly diagnosed severe childhood IgA nephropathy. *J Am Soc Nephrol* 1999; 10: 101–109
14. Pozzi C, Bolasco P, Fogazzi G *et al.* Corticosteroids in IgA nephropathy: a randomized controlled trial. *Lancet* 1999; 353: 883–887
15. Shoji T, Nakanishi I, Suzuki A *et al.* Early treatment with corticosteroids ameliorates proteinuria, proliferative lesions, and mesangial phenotypic modulation in adult diffuse proliferative IgA nephropathy. *Am J Kidney Dis* 2000; 35: 194–201
16. Ballardie FW, Roberts ISD. Controlled prospective trial of prednisolone and cytotoxics in progressive IgA nephropathy. *J Am Soc Nephrol* 2002; 13: 142–148
17. Pozzi C, Andrulli S, Vecchio LD *et al.* Corticosteroid effectiveness in IgA nephropathy: long-term results of a randomized, controlled trial. *J Am Soc Nephrol* 2004; 15: 157–163
18. Katafuchi R, Ikeda K, Mizumasa T *et al.* Controlled, prospective trial of steroid treatment in IgA nephropathy: a limitation of low-dose prednisolone therapy. *Am J Kidney Dis* 2003; 41: 972–983
19. Katafuchi R, Kiyoshi Y, Oh Y *et al.* Glomerular score as a prognosticator in IgA nephropathy: its usefulness and limitation. *Clin Nephrol* 1998; 49: 1–8
20. Taal MW, Brenner BM. Predicting initiation and progression of chronic kidney disease: developing renal risk scores. *Kidney Int* 2006, 70: 1694–1705
21. Katafuchi R, Oh Y, Hori K *et al.* An important role of glomerular segmental lesions on progression of IgA nephropathy: a multivariate analysis. *Clin Nephrol* 1994; 41: 191–198
22. Alarmartine E, Sabatier JC, Guerin C *et al.* Prognostic factors in mesangial IgA glomerulonephritis: an extensive study with univariate and multivariate analysis. *Am J Kidney Dis* 1991; 1: 12–19
23. Donadio JV, Bergstralh EJ, Offord KP *et al.* Clinical and histopathological associations with impaired renal function in IgA nephropathy. *Clin Nephrol* 1994; 41: 65–71
24. D'Amico G, Colasanti G, Barbiano di Belgioioso G *et al.* Long-term follow-up of IgA mesangial nephropathy: clinico-histological study in 374 patients. *Semin Nephrol* 1987; 4: 355–358
25. Kobayashi Y, Tateno S, Hiki Y *et al.* IgA nephropathy: prognostic significance of proteinuria and histological alterations. *Nephron* 1983; 34: 146–153
26. Locatelli F, Pozzi C, Vecchio LD *et al.* Role of proteinuria reduction in the progression of IgA nephropathy. *Ren Fail* 2001; 23: 495–505

Received for publication: 9.9.07
Accepted in revised form: 20.6.08

Clinicopathologic Correlation and Outcome of C1q Nephropathy

Satoshi Hisano,* Yuko Fukuma,† Yoshie Segawa,† Kazuhiko Niimi,† Yoshitsugu Kaku,‡ Ken Hatae,‡ Takao Saitoh,§ Morishige Takeshita,* Hiroshi Iwasaki*

*Department of *Pathology and †Pediatrics, Faculty of Medicine, Fukuoka University, Fukuoka, Japan; §Division of Nephrology and Rheumatology, Faculty of Medicine, Fukuoka University, Fukuoka, Japan; and ‡Department of Nephrology, Fukuoka Municipal Children's Hospital, Fukuoka, Japan*

Background and objectives: The number of patients with C1q nephropathy (C1qN) in previous reports is small and the duration of follow-up is short. Our study describes the clinicopathologic correlation and clinical outcome through the mean follow-up period of 7.2 yr in 61 patients.

Design, settings, participants, & measurements: Sixty-one patients, 1 to 67 yr of age, with C1qN were enrolled in this study.

Results: According to presentation at onset, patients were divided into two groups: asymptomatic urinary abnormalities (asymptomatic) (n = 36) and nephrotic syndrome (NS) (n = 25). Light microscopy showed minimal change disease (MCD) in 46 patients (75%), mesangial proliferative glomerulonephritis in 7 (12%), and focal segmental glomerulosclerosis (FSGS) in 8 (13%). The prevalence of MCD was higher in the NS group than in the asymptomatic group. Nine patients in the asymptomatic group and all patients in the NS group were treated with prednisolone and/or cyclosporine. Normal urinalysis was found in 10 patients in asymptomatic group and 8 in NS group during the follow-up. Thirteen patients in the NS group were frequent relapsers at the latest follow-up. Three patients with FSGS developed chronic renal failure 8 to 15 yr after the diagnosis. C1q deposits disappeared in 3 of 8 patients receiving repeat biopsy, and 2 of these 3 showed FSGS.

Conclusions: The prognosis of C1qN is good, associated with MCD in a large number. In some patients, C1q deposits disappear through the follow-up period. FSGS may develop in some patients on repeat biopsies. Further investigation is critically needed to settle this issue.

Clin J Am Soc Nephrol 3: 1637–1643, 2008. doi: 10.2215/CJN.00830208

C1q nephropathy (C1qN) is a controversial diagnostic entity (1–11). The term was first used by Jennette and Hipp (1,2) in 1985, describing 15 patients with dominant or co-dominant mesangial deposition of C1q on immunofluorescence (IF). Most patients with C1qN were children and young adults. The average age of patients was 10.2 to 24.2 yr (1–12). Previous reports described that the urinary findings in patients with C1qN were heavy proteinuria or nephrotic range proteinuria with or without hematuria (1,3–5,9–11). Iskandar *et al.* (4) reported that the histologic findings were minimal change disease (MCD) in 8 and focal segmental glomerulosclerosis (FSGS) in 7. During the mean follow-up period of 30 mo, clinical outcome was poor in patients with FSGS despite prednisone treatment, and patients with MCD kept normal renal function.

Markowitz *et al.* (9) reported 19 patients with C1qN, 3 to 42 yr of age (mean age, 24.2 yr), presenting nephrotic range proteinuria or nephrotic syndrome. Light microscopy showed FSGS in 17 and MCD in 2. During a mean follow-up period of 27.1 mo, one patient had complete remission of proteinuria and six had partial remission. Four patients with FSGS had progressive renal insufficiency despite steroid and/or immunosuppressive agents. They suggested that C1qN falls within the clinicopathologic spectrum of idiopathic FSGS/MCD.

In our recent report of C1qN in 30 (1.4%) of 2221 children, 1 to 15 yr of age, undergoing renal biopsy, childhood C1qN was found in a wide clinical spectrum showing asymptomatic urinary abnormalities to nephrotic syndrome (NS) (12). A large number of C1qN showed MCD in 73%. The prevalence of FSGS was only 7%. However, FSGS developed in some children on repeat biopsies. There were some children showing the disappearance of C1q deposits through the follow-up period.

The number of patients with C1qN in previous reports is small and the duration of follow-up is short (1–11). A larger number of patients and a longer follow-up study are needed to clarify the clinicopathologic correlation in C1qN. The subjective patients of our previous report were only children (12). The present study here describes the clinicopathologic correlation and clinical outcome through the mean follow-up period of 7.2 yr (3 to 18 yr) in 61 patients, 1 to 67 yr of age, with C1qN, including children and adult patients.

Materials and Methods

Between 1975 and 2004, renal pathology from 16,860 patients, 1 to 76 yr of age, who received percutaneous renal biopsy, was examined at

Received February 19, 2008. Accepted June 20, 2008.

Published online ahead of print. Publication date available at www.cjasn.org.

Correspondence: Dr. Satoshi Hisano, Department of Pathology, Faculty of Medicine, Fukuoka University, 7–45-1 Nanakuma, Jonan-ku, Fukuoka 814-0180, Japan. Phone: 092-801-1011; Fax: 092-863-8383; E-mail: hisanos1@cis.fukuoka-u.ac.jp

ISSN: 1555-9041/306–1637

our laboratory. Sixty-one (0.4%), 1 to 67 yr of age, were diagnosed as C1qN. C1qN was based on the criteria described by Jennette and Falk (3): 1) presence of 2+ or greater of C1q in the mesangium on IF, 2) corresponding mesangial or paramesangial electron dense deposits (EDD) by electron microscopy (EM), and 3) lack of the clinical and pathologic evidence of systemic lupus erythematosus.

Informed consents were obtained from patients or their parents before renal biopsies. After approval was obtained from the Human Ethics Review Committee of Fukuoka University, this study protocol was implemented.

Light microscopy was evaluated on sections stained with periodic acid-Schiff and periodic acid-methenamine silver. Fluorescein isothiocyanate-labeled rabbit anti-human IgG, IgA, IgM, C1q, C3, and fibrinogen polyclonal antibodies (Dako, Copenhagen, Denmark) were used on IF. EM was observed by a JEM 100CX (JEOL, Tokyo, Japan). The degree of interstitial fibrosis was semiquantitatively evaluated on a scale of 0 to 3: 0, no interstitial fibrosis; 1+, 10% to 25% of fibrosis in the interstitium of the cortex; 2+, 26% to 50% of fibrosis in the interstitium of the cortex; 3+, more than 50% of fibrosis in the interstitium of the cortex. The intensity of immunohistologic deposits on IF was semiquantitatively evaluated on a scale of 0 to 4+: 0, negativity of the glomerular area; 1+, almost 25% positivity of the glomerular area; 2+, 26% to 50% positivity of the glomerular area; 3+, 51% to 75% or more positivity of the glomerular area, and 4+, 76% or more positivity of the glomerular area. The intensity of EDD on EM was semiquantitatively scored on a scale of 0 to 3+: 0, no EDD in the glomerular area; 1+, presence of EDD in one part of mesangial, subepithelial, and subendothelial areas; 2+, presence of EDD in two parts of mesangial, subepithelial, and subendothelial areas; 3+, presence of EDD in three or more parts of mesangial, subepithelial, and subendothelial areas. The histology and the grading of IF and EDD were reviewed by 2 observers (S.H. and Y.S.) without prior knowledge of clinical information.

These 61 patients were followed up at affiliated hospitals. The clinical and laboratory parameters examined at the time of the biopsy and at the latest follow-up were blood pressure (BP), urinalysis, serum protein, serum creatinine, serum C3 and C4, antinuclear antibody, anti-DNA, and lupus erythematosus cell preparation. Hematuria and proteinuria were determined as previously reported (13,14). Hematuria was expressed as red blood cells per high-power field in sedimentation and proteinuria was expressed semiquantitatively as milligrams per deciliter. Hypertension in children was defined as BP higher than the 95 percentile for age as indicated by the Task Force on Blood Pressure Control in Children (15). Hypertension in adults was defined according to the National High Blood Pressure Education Program Coordinating Committee (16). NS was defined according to the definition of International Study of Kidney Disease in Children (17) and proteinuria of more than 3.5 g per day plus hypoalbuminemia in adult patients (18). Postural proteinuria was completely excluded in this study.

Prednisolone was administered initially at a dose of 2 mg/kg per day for children or 40 mg per day for adults on each day for 4 wk, followed by a dose of 2 mg/kg per day or 40 mg per day on alternate days for 4 wk, and then tapered off for 4 to 12 mo. The same dose of prednisolone to the initial dose was started at the time of relapse of NS. Cyclosporine was administered at a dose of 2 to 4 mg/kg per day on each day for 6 to 24 mo combined with prednisolone.

Statistical Analysis

Data were expressed as mean ± SD. Association of categorical variables was examined with the χ^2 test. Differences in mean values between groups were examined for statistical significance by using the Mann-Whitney U test.

Results

Profiles of Patients with C1qN

Clinical and pathologic findings at the time of biopsy are listed in Table 1. The mean age was 19.6 ± 15.4 yr. Two groups were classified according to the mode of patients at the time of renal biopsy, as shown in Table 1. Thirty-six patients (59%) were detected as asymptomatic hematuria and/or proteinuria (asymptomatic), and the remaining 25 (41%) were found as NS. In the asymptomatic group, 17 patients (patients 1, 2, 4, 6, 8 to 19, and 21) were detected at the urine screening test for school children and five patients (patients 25, 28, 29, 34, and 35) were detected at the urine screening test in senior high school or university. The remaining 14 patients were by chance found by the urine screening test at a visit to the hospital. None of patients had hypertension at the time of biopsy except for two patients (patients 27 and 30). None of our patients showed the clinical and serologic manifestations associated with systemic lupus erythematosus.

Light microscopic findings were MCD in 46 patients (75%), focal or diffuse mesangial proliferative glomerulonephritis (PGN) in 7 (12%) and FSGS in 8 (13%). Light microscopic findings in asymptomatic group were MCD in 23 (64%), focal or diffuse mesangial PGN in 7 (19%), and FSGS in 6 (17%), whereas those in the nephrotic group were MCD in 23 (92%) and FSGS in 2 (8%). Mild interstitial fibrosis (+) was found in 8 patients (patients 3, 19, 24, 27, 29, 30, 32, and 36) in the asymptomatic group and 2 patients (patients 46 and 61) in the nephrotic group. None of patients showed apparent IgA mesangial deposition. Mesangial deposits of C1q and mesangial EDD were evident in all patients in both children and adults. In the asymptomatic group, mesangial deposits of IgG, IgM, C3, IgG + C3, IgM + C3, and IgG + IgM + C3 were detected in 25 patients (69%), 5 (14%), 21 (58%), 14 (38%), 1 (3%), and 3 (8%), respectively. In the nephrotic group, mesangial deposits of IgG, IgM, C3, IgG + C3, IgM + C3, and IgG + IgM + C3 were found in 13 children (52%), 5 (20%), 1 2 (48%), 8 (32%), 1 (4%), and 1 (4%), respectively.

Mesangial EDD alone, mesangial EDD + subepithelial EDD, mesangial EDD + subendothelial EDD, and mesangial EDD + subepithelial EDD + subendothelial EDD were found in 28 (78%), 5 (14%), 3 (8%), and 1 (3%), respectively, in the asymptomatic group, whereas mesangial EDD alone and mesangial EDD + subendothelial EDD were found in 24 (96%) and 1 (4%), respectively, in the nephrotic group.

Clinical Outcome

Comparison in the clinical findings between the biopsy and the latest follow-up is summarized in Table 2. The mean age at the time of biopsy was not different between the two groups. In renal pathology, the number of MCD was greater in the nephrotic group than in the asymptomatic group. The mean duration of follow-up was 7.2 ± 4.4 yr (range, 3 to 18 yr). The mean duration between the biopsy and the latest follow-up was not different between the two groups. BP was not different at both the biopsy and the latest follow-up between the two groups. The degree of proteinuria was greater at both the biopsy and the latest follow-up in the nephrotic group com-

Table 1. Clinical and pathologic findings in patients with C1q nephropathy

Patient No.	Clinical Findings						LM Findings	IF Findings				EM Findings			Treatment	Follow-up (yr)	Outcome
	Age (yr)	Sex	BP (mmHg)	Hematuria (RBC/HPF)	Proteinuria (mg/dl)	Ccr (ml/min)		IgG	IgM	C1q	C3	Mesangial	Subepithelial	Subendothelial			
Asymptomatic																	
1	11	f	110/60	10	30	146	MCD	2	0	3	2	1	0	0		18	H
2	6	m	96/56	5	30	116	PGN	2	0	3	2	1	0	0		17	H + P
3	3	m	82/36	0	160	72	FSGS	0	1	3	0	1	0	0	Pr	15	CAPD
4	12	m	120/70	70	50	154	MCD	0	0	3	2	1	0	0		15	Normal
5	3	f	78/40	60	150	92	PGN	0	0	1	0	1	0	1	Pr	5	H + P
6	13	f	118/68	0	300	108	MCD	3	0	4	2	2	0	0		3	P
7	5	m	92/46	15	80	86	PGN	3	0	4	2	3	1	1	Pr	4	H + P
8	15	f	128/78	10	100	139	PGN	3	0	4	3	2	0	0		2	P
9	15	f	126/80	20	100	139	MCD	2	0	3	2	1	0	0		3	H + P
10	10	m	112/62	80	40	123	PGN	2	0	3	0	1	0	0		2	H
11	10	f	106/50	10	30	127	MCD	0	0	2	0	1	0	0		14	H
12	11	m	108/60	10	30	150	MCD	2	0	3	2	2	0	1		4	Normal
13	9	m	98/50	10	40	109	MCD	2	0	3	2	1	0	0		3	Normal
14	13	f	110/58	300	30	132	MCD	2	1	3	2	1	0	0		3	Normal
15	12	m	100/52	30	30	159	MCD	2	0	3	0	1	0	0		4	Normal
16	13	f	118/56	50	30	161	MCD	0	0	2	0	1	0	0		3	Normal
17	15	f	126/74	10	40	110	MCD	2	0	3	0	2	1	0		6	Normal
18	6	m	98/40	50	200	98	PGN	2	0	3	0	2	1	0	Pr	4	Normal
19	14	f	126/80	10	400	112	FSGS	0	0	2	0	1	0	0	Pr	18	P
20	3	m	88/40	0	30	78	MCD	2	0	3	0	1	0	0		3	Normal
21	11	f	124/68	100	200	120	MCD	2	1	3	2	1	0	0		3	P + H
22	54	f	130/80	10	40	93	MCD	2	0	3	0	1	0	0		10	H + P
23	30	m	120/70	10	80	156	MCD	0	1	3	2	2	1	0		10	P
24	67	f	138/80	15	0	106	MCD	0	0	3	2	1	0	0		3	H + P
25	17	m	120/56	300	30	116	MCD	0	0	2	0	1	0	0		7	H
26	29	f	126/80	50	40	90	PGN	2	0	3	2	1	0	1		16	H
27	46	f	160/90	0	200	74	FSGS	0	0	3	2	1	0	0	Pr	8	CRF
28	16	f	128/60	300	30	123	MCD	0	0	2	0	1	0	0		6	Normal
29	17	m	124/60	0	150	136	FSGS	2	1	3	2	3	1	0	Pr	6	H + P
30	57	f	160/90	30	250	78	MCD	2	0	3	2	1	0	0		5	H + P
31	32	f	114/70	5	50	77	MCD	2	0	3	0	1	0	0		5	H + P
32	32	m	116/60	10	100	104	FSGS	2	0	3	2	1	0	0	Pr	15	P
33	50	f	135/80	20	200	113	MCD	2	0	3	2	1	0	0		10	P
34	20	f	100/60	100	30	139	MCD	2	0	3	2	1	0	0		12	H
35	16	m	126/66	200	30	132	MCD	2	0	3	0	1	0	0		10	H + P
36	40	f	135/80	15	600	108	FSGS	2	0	3	2	2	0	0	Pr	3	H + P
Nephrotic																	
37	13	m	112/62	0	300	188	MCD	0	0	2	0	1	0	0	Pr	12	Frequent
38	6	m	100/48	0	800	116	MCD	2	0	4	2	2	0	0	Pr+CyA	3	Frequent
39	15	f	128/66	0	700	135	MCD	2	0	3	2	1	0	0	Pr	3	Frequent
40	13	f	120/62	0	200	159	MCD	0	1	4	0	1	0	0	Pr	3	Normal
41	13	m	118/56	10	300	175	MCD	2	0	3	2	1	0	0	Pr	3	Frequent
42	11	m	108/58	0	300	165	MCD	2	0	3	2	1	0	0	Pr	3	Frequent
43	4	m	80/36	0	200	87	MCD	2	0	3	2	1	0	0	Pr	3	Frequent
44	8	m	112/48	0	320	165	MCD	2	0	3	0	1	0	0	Pr+CyA	3	P