



ORIGINAL ARTICLE

Characteristics of patients with coexisting DNAJB9-associated fibrillary glomerulonephritis and IgA nephropathy

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ABSTRACT

Background. Coexistence of fibrillary glomerulonephritis (FGN) and immunoglobulin A (IgA) nephropathy (IgAN) in the same kidney biopsy (FGN–IgAN) is rare, and the clinicopathologic characteristics and outcome of this dual glomerulopathy are unknown.

Methods. In this study, 20 patients with FGN–IgAN were studied and their characteristics were compared with 40 FGN and 40 IgAN control patients.

Results. Concurrent IgAN was present in 1.8% of 847 consecutive FGN cases and was the second most common concurrent glomerulopathy after diabetic nephropathy. FGN–IgAN patients were overwhelmingly White (94%) and contrary to FGN patients were predominantly (60%) males. Compared with IgAN patients, FGN–IgAN patients were older, had higher proteinuria, a higher incidence of renal insufficiency, and a lower incidence of microhematuria and gross hematuria at diagnosis. Six (30%) patients had malignancy, autoimmune disease or hepatitis C infection, but none had a secondary cause of IgAN or clinical features of Henoch–Schönlein purpura. Histologically, all cases exhibited smudgy glomerular staining for immunoglobulin G and DnaJ homolog subfamily B member 9 (DNAJB9) with corresponding fibrillary deposits and granular mesangial staining for IgA with corresponding mesangial granular electron-dense deposits. On follow-up (median 27 months), 10 of 18 (56%) FGN–IgAN patients progressed to end-stage kidney disease (ESKD), including 5 who subsequently died. Serum creatinine at diagnosis was a poor predictor of renal survival. The proportion of patients reaching ESKD or died was higher in FGN–IgAN than in IgAN. The median Kaplan–Meier ESKD-free survival time was 44 months for FGN–IgAN, which was shorter than IgAN (unable to compute, $P = 0.013$) and FGN (107 months, $P = 0.048$).

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Conclusions. FGN-IgAN is very rare, with clinical presentation and demographics closer to FGN than IgAN. Prognosis is guarded with a median renal survival of 3.6 years. The diagnosis of this dual glomerulopathy requires careful evaluation of immunofluorescence findings, and electron microscopy or DNAJB9 immunohistochemistry.

Keywords: DNAJB9, electron microscopy, fibrillary deposits, fibrillary glomerulonephritis, glomerulonephritis, IgA nephropathy

INTRODUCTION

Fibrillary glomerulonephritis (FGN) is a rare primary glomerular disease historically defined by glomerular deposition of Congo red negative, randomly oriented straight fibrils that lack a hollow center and stain with immunoglobulin G (IgG) by immunofluorescence (IF) [1–3]. The recent identification of DnaJ homology subfamily B member 9 (DNAJB9) as a highly sensitive and specific marker for FGN has revolutionized the diagnosis of this disease [4–6] and now allows for distinction from amyloidosis (including the distinction between congophilic FGN and amyloidosis) [7, 8], immunotactoid glomerulopathy [4, 8] and other glomerulopathies characterized by organized deposits [4]. Most reported patients with FGN are White [2, 3, 8, 9] and the disease is diagnosed more frequently in North America (0.6–1.4% of native kidney biopsies) [1, 3, 9–11] than in other continents (0.03–0.4%) [11–13], possibly due to routine performance of electron microscopy (EM) on native kidney biopsies in North America and/or due to genetic or environmental factors [14]. FGN mainly affects middle-aged patients, with a reported average age at diagnosis ranging from 49 to 61 years [8, 9], with approximately a 2:1 female-to-male predominance [8, 9, 11]. It was initially considered to be an idiopathic disease but subsequent studies highlighted association in some cases with autoimmune disease, solid malignancy or hepatitis C [2, 15, 16]. Most patients present with hematuria, proteinuria and renal insufficiency. Prognosis is poor, with about half of patients progressing to end-stage kidney disease (ESKD) within 2–4 years from diagnosis [2, 8]. There is currently no effective therapy, aside from kidney transplantation.

Immunoglobulin A (IgA) nephropathy (IgAN) is the most common primary glomerulonephritis (GN) worldwide, with the highest frequency in East Asians and Whites. It accounts for 40% of glomerular disease diagnoses in Asia, 22% in Europe and 12% in North America [11]. IgAN is defined immunohistologically by IgA-dominant or -codominant glomerular deposits [17]. The IgA staining should be $\geq 1+$ in intensity [18]. The deposits in IgAN are primarily localized to the mesangium and appear granular (i.e. without substructure) on EM. The light microscopy (LM) findings are variable and range from no or minimal mesangial alterations to mesangial proliferative GN to crescentic GN. The Oxford Classification of IgAN was introduced in 2009 and updated in 2017 to standardize the grading of LM features of IgAN, and is currently widely used [18, 19]. Contrary to FGN, there is an approximately a 2:1 male-to-female predominance in North America [11]. While patients with IgAN can present at any age, there is a peak incidence in the second and third decades of life. The clinical presentation is variable and ranges from recurrent gross hematuria, to microscopic hematuria and mild proteinuria, to, rarely, rapidly progressive GN [20]. Prognosis is variable but better than that for FGN; ~ 10 –20% of patients progress to ESKD within 10 years.

The occurrence of FGN and IgAN in the same patient (FGN-IgAN) is quite rare. Concomitant IgAN was present in 2 of 84 (2.4%) FGN patients included in a single-center series [4] but

not in any of 266 FGN patients included in a multi-institutional cohort reported by Andeen et al. [8]. Thus, the incidence, characteristics and prognosis of this dual glomerulopathy are unknown. Herein, we report the first clinicopathologic series on FGN-IgAN, with the aim of defining its clinical characteristics, pathologic features and outcome, and comparing these characteristics with FGN and with IgAN.

MATERIALS AND METHODS

Patients

We retrospectively reviewed the Renal Biopsy Laboratory archives at the Mayo Clinic, Rochester, from January 2007 through March 2020 and identified 15 patients with a native kidney biopsy diagnosis of FGN-IgAN. Five additional patients with available clinical and follow-up data were identified from the pathology archives of Arkana Laboratories during the same time period and were also included in this study. FGN was defined by glomerular deposition of randomly oriented straight fibrils that lack a hollow center and exhibit smudgy staining for IgG by IF and DNAJB9 positivity by immunohistochemistry. IgAN was defined by granular mesangial IgA staining on IF ($\geq 1+$ in intensity on a scale of 0–3+) with corresponding mesangial electron deposits that exhibit granular texture (i.e. without substructure) on EM. Two patients included in this series were previously reported [4].

To determine the incidence of concurrent IgAN and other glomerulopathies in biopsies of FGN, we reviewed the pathology reports of all 847 consecutive cases of FGN accessioned at the Mayo Clinic Renal Biopsy Laboratory and Arkana Laboratories between 2015 and 2019.

Kidney biopsy evaluation

All renal biopsies were processed according to standard techniques for LM, IF and EM. For LM, all cases were stained with hematoxylin and eosin, periodic acid-Schiff, Masson's trichrome and Jones methenamine silver. For DNAJB9 immunohistochemistry, slides were stained with an anti-DNAJB9 rabbit polyclonal antibody (catalog#PA5-59621, 1/200 titer, Thermo Fisher Scientific, Waltham, MA, USA) on a Ventana BenchMark XT system using Ventana OptiView Universal DAB Detection and OptiView Amplification kits. Standard IF on frozen tissue was performed on 3–4 μm cryostat sections using polyclonal fluorescein isothiocyanate (FITC)-conjugated antibodies to IgG, IgM, IgA, C3, C1q, kappa, lambda, fibrinogen and albumin (Dako Corporation, Carpinteria, CA, USA, for the Mayo cases; Kent Laboratories, Bellingham, WA, USA, for the Arkana cases). IF staining for IgG subclass was performed on 3–4 μm cryostat sections using monoclonal FITC-conjugated antibodies to IgG1, IgG2, IgG3 and IgG4 (Sigma-Aldrich Corp., St Louis, MO, USA).

Clinical data

Demographics, renal characteristics, laboratory findings, treatment and follow-up were obtained from patients' medical records. Quantification of proteinuria was performed by 24-h collection or by spot urine protein-to-creatinine ratio when 24-h urine collection was not performed. The following clinical definitions were used: nephrotic-range proteinuria (NRP): ≥ 3.0 g/day; hypoalbuminemia: serum albumin < 3.5 g/dL; renal insufficiency: serum creatinine > 1.2 mg/dL; and nephrotic syndrome: NRP with hypoalbuminemia and peripheral edema. Tubular atrophy and interstitial fibrosis were graded based on an estimate of the percentage of renal cortex affected and recorded as: 0 (none), 1–25% (mild), 26–50% (moderate) or $> 50\%$ (severe). The following outcome definitions were used: (i) remission: reduction in proteinuria by at least 50% with stable renal function (no more than 20% increase in serum creatinine); (ii) persistent renal dysfunction (PRD): failure to meet criteria for remission but not reaching ESKD; and (iii) ESKD: requiring renal replacement therapy. Institutional review board approval was obtained for this study, and it was conducted in accordance with the Declaration of Helsinki.

Control groups

We retrospectively reviewed the Renal Biopsy Laboratory archives at the Mayo Clinic, Rochester, during the same study period and randomly selected 40 Mayo Clinic patients with FGN and 40 Mayo Clinic patients with IgAN in whom complete clinical and follow-up data were available as two control groups at a ratio of 2:1.

Statistical analyses

Data were analyzed using SPSS Statistics, version 17.0 (Chicago, IL, USA) and StatXact/LogXact, version 11 (Cytel Software Corp., Cambridge, MA, USA). The one-sample Kolmogorov–Smirnov test was used to determine the normality of distribution. Continuous variables are reported as mean \pm standard deviation or, for non-normally distributed variables, as median (25–75% interquartile range). Analysis was performed using non-parametric exact statistical methods using the Mann–Whitney–Wilcoxon test, the Kruskal–Wallis test, the Jonckheere–Terpstra test and the Fisher–Freeman–Halton exact test, as appropriate for variable type. Survival analysis for progression to ESKD was performed by using the Kaplan–Meier method for categorical variables and by Cox regression for continuous variables. Multivariate survival analyses were performed using the Cox proportional hazards model (Cox regression). Statistical significance was assumed at $P < 0.05$.

RESULTS

Clinical features

The demographics, renal characteristics and outcome for each of the 20 patients with FGN–IgAN are listed in [Table 1](#), and their pathologic characteristics are listed in [Table 2](#). [Table 3](#) compares the pertinent clinicopathologic features and outcome of patients with FGN–IgAN with those of FGN and those of IgAN. The FGN–IgAN cohort was overwhelmingly White (94%), with male predominance (60%). The male predominance was in contrast to FGN patients, in whom 70% were females ($P = 0.049$), but not different from IgAN patients [68% males, $P =$ not significant (NS)]. The mean age at diagnostic biopsy for FGN–IgAN was

57 years (range 35–78). Seven (35%) were elderly (≥ 64 years of age). FGN–IgAN patients were older than IgAN patients ($P = 0.03$). Associated conditions included malignancy in 15% (non-Hodgkin's lymphoma in one, prostate cancer in one and throat cancer in one), autoimmune disease in 10% (lupus in one and sarcoidosis in one) and hepatitis C infection (HCV) in one (5%). Coexistent conditions included hypertension in 65%, diabetes mellitus in 25% and hypothyroidism in 10%.

Most patients with FGN–IgAN presented with proteinuria, microhematuria and renal insufficiency. Thus, at the time of diagnostic biopsy, all patients had proteinuria (median 24-h urine protein 3.9 g), which was higher than in patients with IgAN ($P = 0.001$) but not patients with FGN ($P =$ NS). Whereas proteinuria was within the nephrotic range in 65%, only 18% had full nephrotic syndrome. Microhematuria was documented in 79% of patients. No patient presented with recurrent gross hematuria, contrary to IgAN patients (30%, $P = 0.012$). Renal insufficiency at diagnosis was present in 74%; the mean serum creatinine was 1.9 mg/dL (range 0.6–4.9).

Serum complement C3 and C4 were normal in all 16 patients tested. Testing for hepatitis B surface antigen was done in 17 patients and was negative in all, while hepatitis C antibody was positive in only 1 of the 17 patients tested. ANA was positive in 3 of 14 (21%) patients tested. antineutrophil cytoplasmic antibody was negative in all 12 patients tested including one with crescentic GN on biopsy. Serum protein electrophoresis with immunofixation, performed in 18 patients, and urine protein electrophoresis with immunofixation, performed in 11, were negative for monoclonal protein.

Pathologic findings

Light microscopy. Sampling for LM included a mean of 20 glomeruli, a mean of 39% of which were globally sclerotic. The percentage of global glomerulosclerosis (GGS) was higher than in FGN ($P = 0.037$) and IgAN ($P = 0.018$). The most common pattern of glomerular injury was by far mesangial proliferative/sclerosing glomerulonephritis (MesGN), observed in 18 (90%) cases, with variable degrees of mesangial hypercellularity, sclerosis and expansion by immune deposits ([Figure 1A and Table 2](#)). One case (5%, Case #17 in [Table 2](#)) showed focal endocapillary proliferative GN. This pattern was less common than in patients with IgAN ($P = 0.043$). The remaining case (5%, Case #1 in [Table 2](#)) showed crescentic and necrotizing GN with crescents involving 24% of glomeruli (62% of nonsclerotic glomeruli) and fibrinoid necrosis involving 14% of nonsclerotic glomeruli. Two cases with MesGN pattern showed concurrent nodular (one case) or diffuse (one case) diabetic glomerulosclerosis. In addition to the one case of crescentic GN, three cases showed focal crescents involving 10, 9 and 4% of glomeruli. The degree of tubular atrophy and interstitial fibrosis (TA/IF) ranged from absent in 2 cases to mild in 10, to moderate in 5 and to marked in 3. Arteries were sampled in 19 cases. The degree of arteriosclerosis ranged from none in six cases to mild in six, to moderate in six and to marked in one. Glomeruli showed strong smudgy staining for DNAJB9 in all 20 cases ([Figure 1B](#)). Congo red stain was negative in all 15 cases tested.

Immunofluorescence. On standard IF on frozen tissue, all cases showed granular mesangial staining for IgA with a median intensity of 2+ (on a scale of 0–3+; [Figure 1C](#)) and smudgy mesangial and glomerular capillary wall (GCW) staining for IgG with a median intensity of 2+ ([Figure 1D](#) and [Tables 2 and 3](#)). The intensity of IgG staining was lower in FGN–IgAN than in FGN

Table 1. Demographics, renal characteristics and outcome of 20 patients with FGN-IgAN

Case number	Age (years)	Gender	Race	S. Cr. (mg/dL)	Proteinuria (g/day)	S. albumin	Edema	Full nephrotic syndrome	Hematuria	Concurrent conditions	F/U duration in months	Treatment	Outcome	S. Cr. (g/day)	Last F/U proteinuria ESKD in months	Last F/U Time to ESKD in months
1	66	M	W	4.9	5.1	4.5	No	No	Yes	None	14	Steroids, rituximab	ESKD	ESKD	ESKD	3
2	64	M	W	0.9	1.7	3.6	No	No	Yes	HTN, prostate cancer	95	None	ESKD	ESKD	ESKD	52
3	54	M	W	1.3	4.1	4	Yes	No	No	HTN	123	Steroids	ESKD/ death	ESKD	ESKD	65
4	35	M	W	0.9	2.3	3.6	No	No	Yes	None	20	Cyclophosphamide, rituximab	remission	1	0.285	
5	57	M	W	4	7.7	3.4	No	No	Yes	HTN, DM	21	None	ESKD/ death	ESKD	ESKD	8
6	60	F	W	3.6	1	3.7	No	No	Yes	HTN, non-Hodgkin lymphoma	44	Steroids	ESKD/ death	ESKD	ESKD	44
7	78	F	W	2	3.9	4.2	No	No	Yes	HTN	13	ARB (losartan)	Remission	1.5	0.039	
8	53	M	W	2.1	4.1	NA	NA	NA	Yes	none	133	Steroids	ESKD/ death	ESKD	ESKD	131
9	46	F	W	0.7	0.86	4.5	Yes	No	Yes	None	14	ACEI (lisinopril), fish oil	Remission	0.7	0.27	
10	64	M	W	1.7	7.3	3	Yes	Yes	Yes	HTN, DM, ischemic cardiomyopathy	25	MMF	ESKD	ESKD	ESKD	22
11	53	M	W	1.3	2.4	4.2	Yes	No	Yes	Fatty liver, obesity	5	ACEI (lisinopril), ARB (losartan)	Remission	1.3	0.8	
12	51	M	H	1	3.1	4.1	No	No	No	HTN, lupus	35	None	PRD	1.4	0.983	
13	58	M	W	2.4	14	3	Yes	Yes	Yes	HTN, DM	38	CyBorD	ESKD/ death	ESKD	ESKD	23
14	49	M	W	2.1	0.75	3.2	No	No	Yes	HCV, IV drug use	28	Zepatier (elbasvir and garzoprevir) for HCV	PRD	4	13.6	
15	69	F	W	2.6	6.7	2	No	No	Yes	HTN	29	ARB (losartan)	ESKD	ESKD	ESKD	7
16	74	F	NA	1.7	5.6	NA	No	No	Yes	HTN, nonischemic cardiomyopathy	NA					
17	41	F	W	1.5	3.8	NA	Yes	NA	No	HTN, sarcoidosis, hypothyroidism	15	ARB (temisartan)	PRD	1.6	2.8	
18	73	F	W	NA	9	Low	Yes	Yes	No	HTN, DM	10	Steroids, MMF, rituximab	NA	1.3	NA	
19	43	M	W	3.5	3.8	NA	NA	NA	NA	HTN, DM, throat cancer	84	NA	ESKD	ESKD	ESKD	12
20	45	F	NA	0.6	1.6	NA	No	No	Yes	Hypothyroidism	NA					

ACE I, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; CyBorD, cyclophosphamide+bortezomib+dexamethasone; DM, diabetes; F, female; F/U, follow-up; H, Hispanic; HTN, hypertension; IV, intravenous; M, male; NA, not available; S. albumin, serum albumin; S. Cr., serum creatinine; W, White.

Table 2. Pathologic findings by LM, IF and EM in patients with FGN-IgAN

Case number	LM	Pattern of injury	Number of glomeruli in LM	% GGS	% crescents	TA/IF	IF ^a	IgG	IgA	IgM	C1q	C3	κ	λ	EM	Mes. fibrillary deposits	GBM fibrillary deposits	Mes. granular electron-dense deposits	GCW granular electron-dense deposits	Mean fibril thickness in nm	FPE
1		Crescentic GN	33	61	24	Mild	3+	1+	2+	0.5+	0	2+	0	2+	2+	Yes	Yes (G)	Yes	Yes	15	G
2		MesGN	24	0	0	Mild	1+	1.5+	1.5+	1.5+	0	1+	0.5+	1.5+	1.5+	Yes	Yes (S)	No	No	17	S
3		MesGN	20	30	10	Mild	0.5+	1+	0	0	2+	2+	1+	2+	2+	Yes	Yes (S)	No	No	19	G
4		MesGN	19	37	0	Mild	2+	2+	1+	0.5+	2+	2+	1+	2+	2+	Yes	Yes (G)	No	No	17	S
5		MesGN + nodular DGS	44	66	0	Marked	1+	1.5+	0	1+	1.5+	1+	1+	1+	1+	Yes	Yes (S)	Yes	No	15	G
6		MesGN	11	18	9	Mild	1+	1+	0	0	0	1+	1+	1+	1+	Yes	Yes (S)	No	No	12	S
7		MesGN	14	43	0	Mild	1+	1+	0.5+	0	1.5+	1.5+	1.5+	1+	1+	Yes	Yes (G)	Yes	No	18	S
8		MesGN	5	60	0	Moderate	3+	3+	3+	0.5+	0	3+	0.5+	2+	2+	Yes	Yes (S)	No	No	13	S
9		MesGN	36	6	0	None	2.5+	1.5+	0.5+	0.5+	2.5+	2.5+	1.5+	1+	1+	Yes	Yes (G)	Yes	No	21	G
10		MesGN + DGS	3	67	0	Moderate	1+	1+	1+	0	0	2+	1+	1+	1+	Yes	Yes (G)	No	No	14	S
11		MesGN	7	14	0	Mild	2+	2+	2+	0.5+	2+	2+	1.5+	2+	2+	Yes	Yes (S)	Yes	No	13	S
12		MesGN	13	62	0	Mild	1.5+	2+	1.5+	1+	2+	2+	1+	2+	2+	Yes	Yes (S)	No	No	15	G
13		MesGN	16	50	0	Marked	2+	1+	0	0.5+	2+	2+	1+	0.5+	0.5+	Yes	Yes (G)	Yes	Yes	16	S
14		MesGN	27	15	4	Mild	1+	2.5+	0.5+	0	2.5+	2.5+	0.5+	1+	1+	Yes	Yes (S)	No	No	NM	G
15		MesGN	50	80	0	Marked	2.5+	1.5+	1.5+	1+	1+	3+	1+	1.5+	1.5+	Yes	Yes (G)	Yes	Yes	17	G
16		MesGN	10	90	0	Moderate	2+	3+	0	0	2+	2.5+	2+	3+	3+	Yes	Yes (S)	No	No	17	G
17		EPGN	15	33	0	Moderate	3+	2+	0.5+	0.5+	1.5+	1.5+	2+	2+	2+	Yes	Yes (S)	No	No	17	G
18		MesGN	23	8	0	Moderate	3+	3+	0	0	0	0	3+	3+	3+	Yes	Yes (S)	No	No	19	S
19		MesGN	7	43	0	Mild	3+	2+	0	0	2+	2+	2+	2+	2+	Yes	Yes (S)	Yes	No	20	S
20		MesGN	18	0	0	None	3+	3+	1+	1+	1+	3+	3+	3+	3+	Yes	Yes (S)	Yes	Yes	17	S

DGS, diabetic glomerulosclerosis; EPGN, endocapillary proliferative glomerulonephritis; FPE, degree of podocyte foot process effacement; G, global; Mes., mesangial; NM, not measured; S, segmental. ^aIF intensities scale: 0-3+.

Table 3. Comparison of clinical and pathologic characteristics among FGN-IgAN, FGN and IgAN

Characteristics	FGN-IgAN	FGN	IgAN	P (FGN-IgAN vs FGN vs IgAN)	P (FGN-IgAN vs FGN)	P (FGN-IgAN vs IgAN)
Number of patients	20	40	40			
Clinical characteristics						
at diagnosis						
Age in years	56.7 ± 11.8	55.7 ± 11.5	46.1 ± 18.8	0.019	NS	0.033
Gender (M:F)	12:8	12:28	27:13	0.003	0.049	NS
White race	17/18	38/40	35/40	NS	NS	NS
Proteinuria (g/day)	3.85 (1.85, 6.42)	3.00 (1.65, 7.25)	1.55 (0.57, 3.08)	<0.001	NS	0.001
Serum albumin (g/dL)	3.64 ± 0.69	3.33 ± 0.72	3.83 ± 0.53	0.012	NS	NS
Edema	7/18	19/40	8/40	0.023	NS	NS
Nephrotic syndrome	3/17	10/40	1/40	0.008	NS	NS
S. Cr. (mg/dL)	1.70 (1.00, 2.60)	1.40 (0.83, 1.80)	1.65 (1.20, 2.18)	NS	NS	NS
Renal insufficiency	14/19	22/40	27/40	0.013	0.052	0.005
Microhematuria	15/19	30/39	39/40	0.011	NS	0.033
Gross hematuria	0/16	2/40	12/40	0.001	NS	0.012
Hypertension	13/20	22/40	23/40	NS	NS	NS
Hypocomplementemia	0/16	1/31	2/35	NS	NS	NS
Concurrent conditions						
Diabetes	5/20	6/40	6/40	NS	NS	NS
Autoimmune disease	2/20	9/40	8/40	NS	NS	NS
HCV	1/20	4/40	0/40	NS	NS	NS
Malignancy	3/20	8/40	2/40	NS	NS	NS
Monoclonal gammopathy	0/15	1/40	5/40	NS	NS	NS
Pathologic characteristics						
Pattern of glomerular injury on LM				NS	NS	NS
MesGN	18	32	24	0.023	NS	0.019
EPGN	1	1	12	0.001	NS	0.043
Crescentic GN	1	3	1	NS	NS	NS
MPGN	0	3	1	NS	NS	NS
Unremarkable	0	1	2	NS	NS	NS
% GGS on LM	39.2 ± 27.4	23.9 ± 17.8	21.7 ± 17.9	0.036	0.037	0.018
Presence of cellular or fibrocellular crescents	4/20	6/40	12/40	NS	NS	NS
Tubular atrophy and interstitial fibrosis (none, mild, moderate and marked)	2:10:5:3	1: 27: 9: 3	6: 26: 7: 1	NS	NS	NS
Arteriosclerosis (none, mild, moderate and marked)	6: 6: 6: 1	1: 27: 9: 3	13: 14: 9: 3	NS	NS	NS
IgG staining intensity ^a	2.0 (1.0–3.0)	3.0 (2.5–3.0)	0.0 (0.0–0.0)	<0.001	0.001	<0.001
IgA staining intensity ^a	2.0 (1.1–2.4)	0.0 (0.0–0.4)	2.5 (2.0–3.0)	<0.001	<0.001	0.022
IgM staining intensity ^a	0.5 (0.0–1.0)	0.0 (0.0–0.5)	0.5 (0.1–1.0)	0.047	NS	NS
C3 staining intensity ^a	2.0 (1.5–2.4)	2.0 (1.6–3.0)	2.0 (1.0–3.0)	NS	NS	NS
C1q staining intensity ^a	0.0 (0.0–0.5)	0.0 (0.0–0.5)	0.0 (0.0–0.0)	0.003	NS	0.001
Kappa staining intensity ^a	1.0 (1.0–1.9)	2.0 (1.5–3.0)	1.0 (1.0–2.0)	0.004	0.007	NS
Lambda staining intensity ^a	2.0 (1.0–2.0)	2.0 (1.0–2.5)	2.0 (1.5–3.0)	NS	NS	NS
Follow-up						
Duration of follow-up in months	27 (14–54)	28.5 (19–46)	27 (15–36)	NS	NS	NS
IS therapy (steroids with/without other IS agents)	8/18	22/40	13/40	NS	NS	NS
ESKD	10/18	11/40	4/40	0.001	NS	<0.001
Death	5/18	3/40	1/40	0.02	NS	0.009

EPGN, endocapillary proliferative glomerulonephritis; F, female; M, male; MPGN, membranoproliferative glomerulonephritis; NS, not significant. Values are presented as numbers, mean ± standard deviation for continuous variables and median (25–75% interquartile range) for non-normally distributed continuous variables.

^aIF staining intensity on a scale of 0–3+.

($P=0.001$) and expectedly higher than in IgAN ($P<0.001$), and the intensity of IgA staining was lower in FGN-IgAN than in IgAN ($P=0.022$) and expectedly higher than in FGN ($P<0.001$; Table 3). Glomerular deposition of C3 was detected in 95% of

cases (median intensity 2+), IgM in 60% (median intensity 0.5+) and C1q in 45% (median intensity 0). All cases, except one, showed staining for both lambda light chain (median intensity 2+) and kappa light chain (median intensity 1+). The one

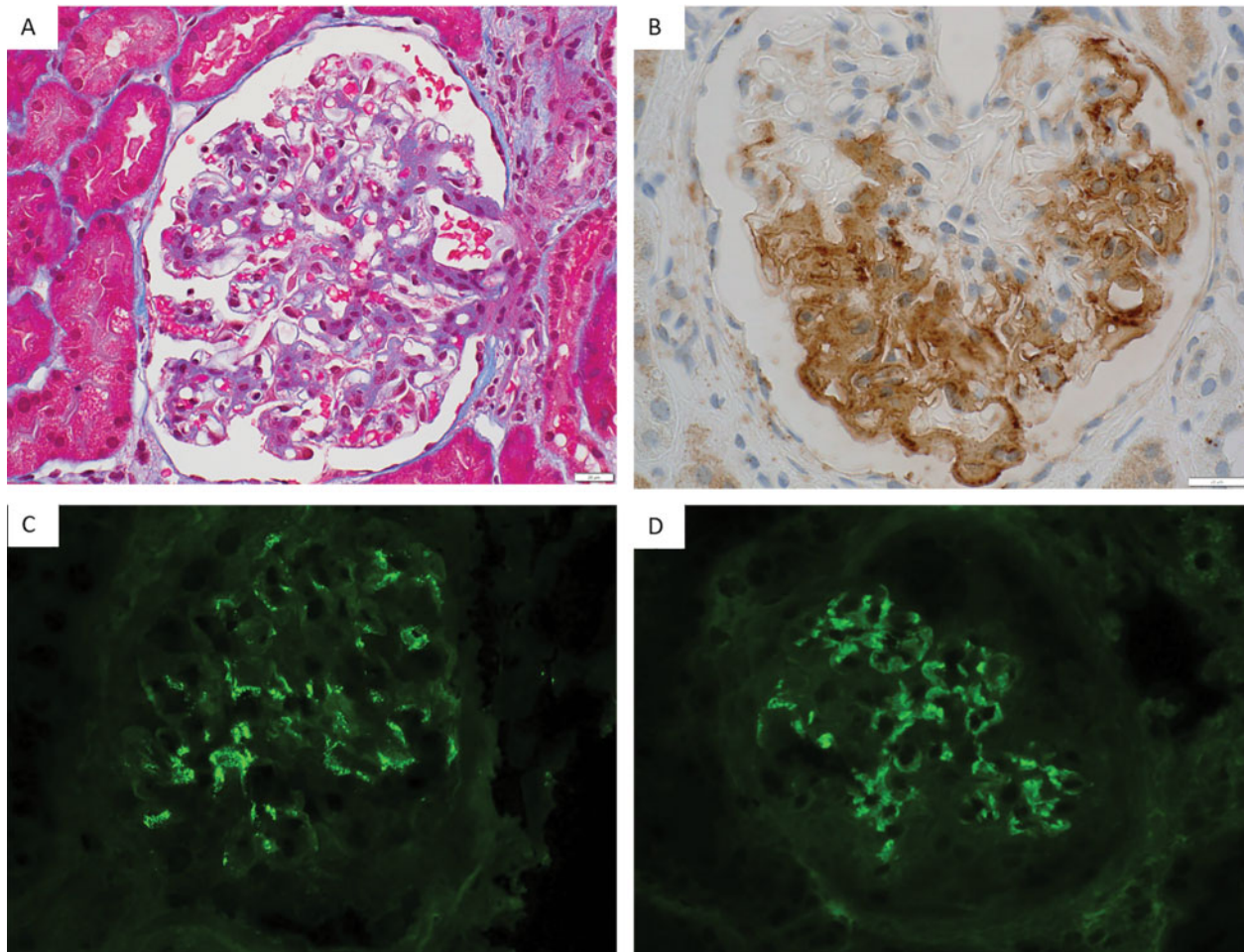


FIGURE 1: Pathologic findings on LM and IF (A) A representative glomerulus from Case #3 showing mild global mesangial hypercellularity and matrix expansion (trichrome stain, $\times 400$). (B) A representative glomerulus from Case #2 showing smudgy mesangial and GBM positivity for DNAJB9 immunohistochemical stain ($\times 600$). (C) A representative glomerulus from Case #6 showing granular global mesangial staining for IgA ($\times 400$). (D) A representative glomerulus from the same case (#6) exhibiting smudgy mesangial and GBM staining for IgG ($\times 400$).

exception (Case #1) stained 2+ for lambda with negative staining for kappa; however, by IF on paraffin tissue after pronase digestion, it stained for both lambda (3+) and kappa (1+), and this patient had negative serum immunofixation and normal serum-free light-chain ratio. Focal extraglomerular staining for IgG was present in 15% of cases (involving tubular basement membranes in two patients and arterioles in one). IF staining for IgG subtypes, performed on 11 cases, showed heavy chain isotype restriction (i.e. staining for a single IgG subclass) in six (55%, IgG4 in three and IgG1 in three) and staining for more than one subclass in the remaining five cases (IgG4 dominant in two, IgG1 dominant in two and IgG1 and IgG4 codominance in one).

Electron microscopy. All cases exhibited randomly oriented straight fibrils (mean thickness 16 nm, range 12–21 nm) permeating the mesangial matrix and the glomerular basement membranes (GBM), typical of FGN (Figure 2A and B; Table 2). The GBM fibrillar deposits were segmental (i.e. involving <50% of GCW) in 13 cases and global in the remaining 7 cases. In addition to the fibrillar deposits, all cases exhibited mesangial granular electron-dense deposits (i.e. without substructure). In contrast to the mesangial fibrillar deposits that intermingled with the mesangial matrix, the mesangial granular electron-dense deposits in most cases formed discrete deposits with a

tendency to localize beneath the GBM reflection over the mesangium (so referred to as ‘paramesangial deposits’; Figure 2C and D), as seen in the majority of IgAN cases. Rare, small granular GCW deposits (intramembranous or subendothelial) were seen in four (20%) cases.

Treatment and outcome

Follow-up was available on 18 patients with FGN-IgAN. The median duration of follow-up was 27 months (range 5–133 months). On follow-up, 10 (56%) progressed to ESKD (Table 1). Three of these patients did not receive immunosuppression therapy, three were treated with steroids only, one received steroids and rituximab, one received mycophenolate mofetil (MMF) only and one was treated with seven cycles of cyclophosphamide + bortezomib + dexamethasone. The latter patient initially partially responded to this chemotherapeutic regimen with reduction of proteinuria from 14 to 6 g/day and reduction of serum creatinine from 2.4 to 1.4 mg/dL, but subsequently advanced to ESKD. Data on treatment were not available on the remaining patient. Five patients with ESKD died, one of acute coronary artery disease, one of sepsis in the setting of *Clostridium difficile* colitis, one of pneumonia, one of metastatic lung cancer and one of undetermined cause. One of the patients with ESKD (Case #19) underwent a living-

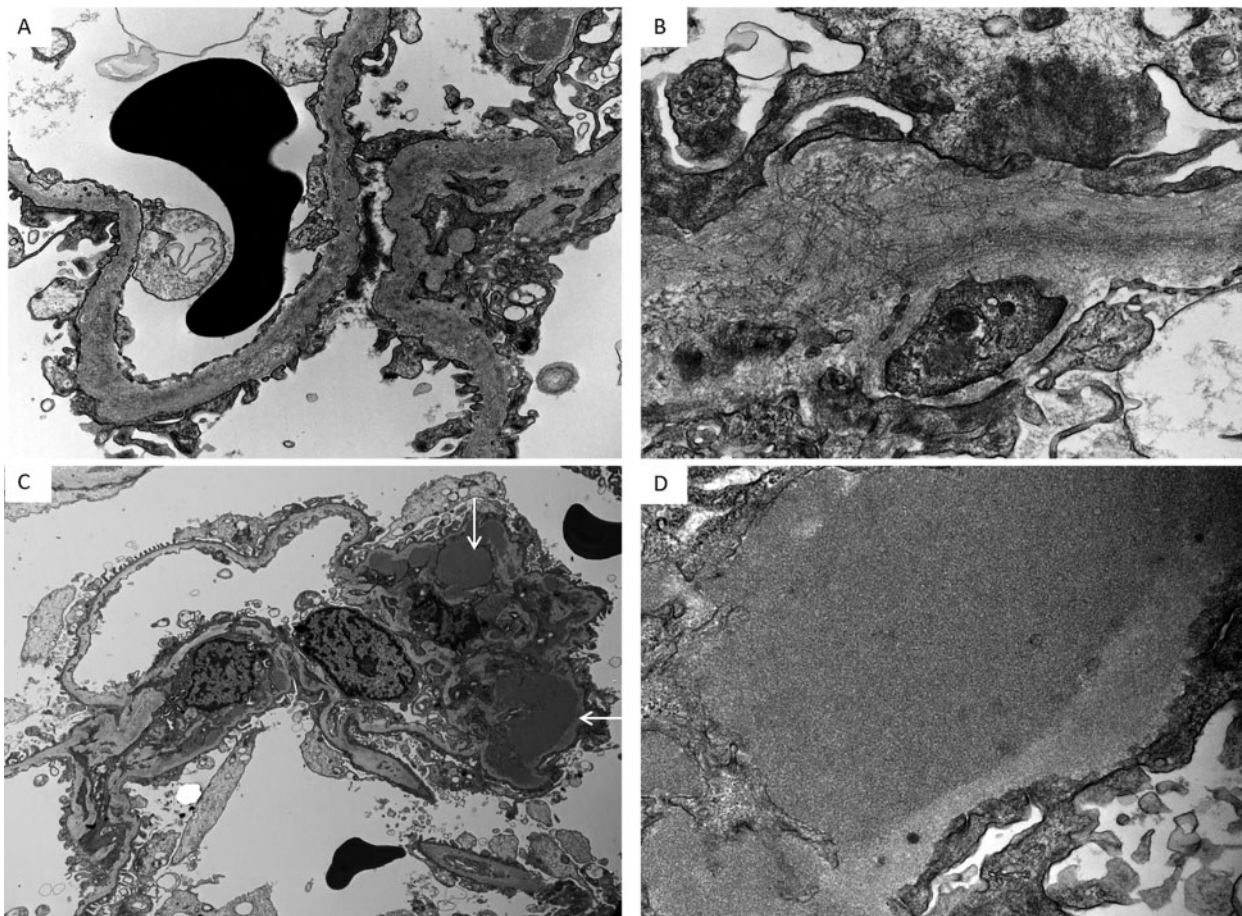


FIGURE 2: EM findings in Case #11 (A) Fibrillary deposits permeating the GBM ($\times 12\,000$). (B) On high magnification, infiltration of the GBM by randomly oriented straight fibrils (mean thickness 14 nm and range 11–18 nm) is evident ($\times 30\,000$). (C) In the same biopsy, there were also discrete electron-dense deposits (arrows) localized beneath the GBM reflection over the mesangium ('paramesangial deposits'; $\times 4\,000$). (D) A higher magnification of the deposits depicted in (C) showing granular texture (i.e. without substructure; $\times 30\,000$).

donor kidney transplant and had biopsy-proven recurrence of IgAN (without FGN) in the renal allograft. Seven-year postimplantation, his proteinuria was 7.5 g/day and serum creatinine was 1.0 mg/dL.

Of the remaining eight (44%) patients who did not progress to ESKD, three had PRD [none of whom received immunosuppressive (IS) agents], four had remission (three of whom did not receive IS agents and one received cyclophosphamide and rituximab) and one had an elevated serum creatinine at last follow-up but the degree of proteinuria was not available (who was treated with steroids, MMF and rituximab; [Table 1](#)).

Using Cox regression, the only predictor of reaching ESKD by univariate analysis in patients with FGN-IgAN was a higher serum creatinine at biopsy ($P = 0.008$). Using the Cox proportional hazards model, the only independent predictor of the rate of progression to ESKD by multivariate analysis was a higher serum creatinine at biopsy ($P = 0.039$; hazard ratio = 4.445; 95% confidence interval 1.077–18.349). Proteinuria at biopsy, serum albumin and degree of GGS, TA/IF and arteriosclerosis did not predict renal survival.

The duration of follow-up and proportion of patients who received IS agents (steroids and/or other IS agents) were not different among the FGN-IgAN, FGN and IgAN groups ([Table 3](#)). The proportion of patients reaching ESKD was higher in FGN-IgAN than in IgAN (56% versus 10%, $P < 0.001$) and tended to be higher than in FGN (28%), but this did not reach statistical significance. Likewise, the death rate in FGN-IgAN was higher

than in IgAN (28% versus 3%, $P = 0.009$) and tended to be higher than in FGN (8%), but this did not reach statistical significance. The median Kaplan–Meier ESRD-free survival time was 44 months for FGN-IgAN, which was shorter than IgAN (unable to compute, $P = 0.013$) and FGN (107 months, $P = 0.048$; [Figure 3](#)).

Repeat native biopsies

Four patients had prior and/or follow-up native kidney biopsies. Patients #4 and #13 had kidney biopsies 5 and 3 years prior to the diagnostic biopsies that showed FGN without IgAN indicating that FGN preceded IgAN in these two patients. Patient #13 also had a third biopsy one and a half years following the diagnostic biopsy which revealed diffuse sclerosing GN with both fibrillary and granular deposits by EM but no glomeruli were sampled for IF.

Patient #8 had a repeat biopsy 11 years following the diagnostic biopsy that showed active IgAN (two of six sampled glomeruli with cellular crescents on LM, bright glomerular staining for IgA on IF and abundant granular deposits on EM) with burnt-out FGN (only rare remnant fibrillary deposits on EM and trace staining for IgG by IF).

Patient #14 had a repeat biopsy 2 years after the diagnostic biopsy that showed diffuse sclerosing GN with evidence of persistent FGN and IgAN on IF (with both smudgy staining for IgG

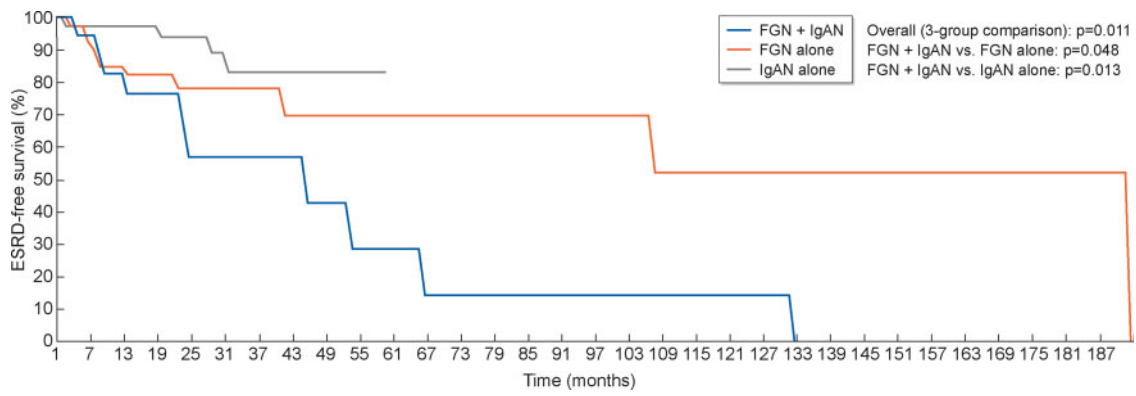


FIGURE 3: Kaplan–Meier ESKD-free survival curve for FGN–IgAN, FGN and IgAN.

Table 4. Concurrent glomerulopathies in 847 consecutive cases of FGN accessioned between 2015 and 2019 at the Mayo Clinic Renal Biopsy Laboratory and Arkana Laboratories

Number of FGN cases	Number of cases with concurrent glomerulopathy (%)	Type of concurrent lesion (%)
847	147 (17.4)	Diabetic glomerulosclerosis = 107 (12.6%) IgA nephropathy = 15 (1.8%) ANCA-associated crescentic GN = 5 (0.6%) Thrombotic microangiopathy = 5 (0.6%) Membranous nephropathy = 4 (0.5%) Smoking and HTN-associated mesangial sclerosing glomerulopathy = 3 (0.4%) Collapsing glomerulopathy = 2 (0.2%) Thin basement membrane disease = 2 (0.2%) Anti-GBM nephritis = 1 (0.1%) Bacterial infection-associated GN = 1 (0.1%) Lupus nephritis class IV = 1 (0.1%) Minimal change disease = 1 (0.1%)

and granular staining for IgA) and EM (with both fibrillary and granular deposits).

DISCUSSION

In our experience, the overall incidence of a concurrent glomerulopathy in FGN is 17.4% (147/847; Table 4). Concurrent IgAN appears to be very rare, present in only 1.8% of FGN cases, but it is the second most common concurrent glomerulopathy after diabetic nephropathy (Table 4) which is not surprising considering the higher frequency of IgAN than other primary glomerular diseases. This study describes the clinical features, pathologic characteristics and outcome of patients with FGN concurrent with IgAN. In contrast to FGN, which is more common in

females [9, 11], FGN–IgAN was more common in males, similar to IgAN in North America [11]. Mean age at diagnosis was 57 years, which was comparable to FGN patients and higher than IgAN patients. FGN–IgAN patients had a higher degree of proteinuria, higher incidence of renal insufficiency and lower incidence of microhematuria at diagnosis than IgAN patients. Notably, no FGN–IgAN patient presented with recurrent macrohematuria accompanying upper respiratory tract infect ‘synpharyngitic hematuria’, which is a common presentation in IgAN in North America [21] and which was observed in 30% in our IgAN control group. Thus, FGN–IgAN has similar renal presentation and demographics (aside from male predominance) to FGN. Overall, 30% (6/20) of patients had underlying HCV, autoimmune disease or malignancy, conditions known to be present in some patients with FGN [2, 15, 16], and thus exclusion of such conditions in patients with FGN–IgAN is important. Noticeably, no patient had liver cirrhosis, celiac disease, inflammatory bowel disease, HIV infection or systemic features of Henoch–Schonlein purpura, favoring that IgAN in these patients is mostly idiopathic and renal limited.

The prognosis of FGN–IgAN seems to be guarded, as over a half of patients progressed to ESKD within a median follow-up of 27 months. The proportion of FGN–IgAN patients reaching ESKD was higher than in IgAN patients, and the ESKD-free survival was shorter in FGN–IgAN than in IgAN and FGN; thus, the accurate diagnosis of this dual glomerulopathy is clinically relevant.

Of note, a repeat native biopsy in one patient showed active IgAN with burnt-out FGN and an allograft biopsy in another patient revealed IgAN without FGN; thus, repeat biopsies might be needed to determine the evolution of this lesion in patients who are clinically suspected to have a disease relapse.

Due to the limited sample size, we cannot draw conclusions about the appropriate therapy for FGN–IgAN. We note, however, that three of the four patients who had disease remission were not treated with IS agents, suggesting that conservative therapy alone may be appropriate for mild cases. Rituximab, which may preserve kidney function in some patients with FGN [8, 22], was given (together with other IS agents) to three patients, one of whom had remission, one had persistently elevated serum creatinine and one progressed to ESKD. Larger studies with longer follow-up are needed to determine the optimal therapy.

The diagnosis of FGN–IgAN requires careful evaluation of IF findings, which will demonstrate dual pattern of glomerular positivity: smudgy mesangial and GCW staining for IgG and granular mesangial staining for IgA. The diagnosis is confirmed by the ultrastructural findings of both randomly oriented fibrils

involving the mesangium and GBM and granular electron-dense deposits that tend to localize in the paramesangial areas. This dual glomerulopathy is likely underdiagnosed as EM is not often available in renal pathology laboratories in developing countries and is not routinely performed in the evaluation of native renal biopsies in the developed countries, including some countries in Europe [23]. Thus, 70% (14/20) of our FGN-IgAN had IgG staining intensity weaker (six cases) or equal to (eight cases) IgA staining intensity, and these cases could be misdiagnosed as IgAN alone if careful evaluation of the pattern of IF staining at high magnification and EM were not performed. The availability of DNAJB9 immunohistochemical stain, a highly sensitive and specific marker for FGN [4–6], now allows for easier diagnosis of FGN. Of note, there have been two reported cases of IgA-dominant FGN [24, 25], which are different than our cases in that they did not exhibit IgG staining on IF or granular electron-dense deposits on EM. Thus, these two cases do not represent FGN-IgAN but rather exceptional cases of an IgA variant of FGN.

Given the different morphology on IF and EM and pathophysiology of these two forms of GN, we favor that the occurrence of FGN and IgAN in the same biopsy likely represents a chance occurrence of two unrelated conditions. Furthermore, all 20 cases were positive for DNAJB9 and, when tested, exhibited IgG4 and/or IgG1 restriction or dominance (as seen in FGN alone), arguing against a different pathogenesis for FGN in these cases. Since IgA deposits without clinical evidence of kidney disease are present in 5–16% of healthy individuals [26, 27], it is tempting to speculate that FGN-IgAN probably represents FGN superimposed on preexisting mild IgAN. However, in two of our FGN-IgAN patients prior biopsies showed FGN without IgAN, indicating that in at least some patients, the development of FGN precedes that of IgAN.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no relevant financial interests. The results presented in this article have not been published previously in whole or part.

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