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Clinical Characteristics of Nephrin Autoantibody-Positive Minimal Change Disease in Older Adults

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INTRODUCTION

Minimal change disease (MCD) is a major cause of the adult-onset nephrotic syndrome, particularly in Japan where it affects 40% of patients compared with 15% of patients worldwide.[1] Its incidence is particularly high among the elderly and both the disease and corticosteroid therapy can be a major cause of morbidity in this population.

Although the pathogenesis of MCD remains to be fully elucidated, the involvement of B- and T-cells has been known for many years,[1] and more recently, evidence for autoantibodies directed against podocyte antigens in the pathogenesis has been proposed.

In a recent study by Watts *et al.*,[2] autoantibodies against the critical slit diaphragm protein nephrin were identified in the serum and glomeruli of a North American cohort with biopsy-proven MCD strongly suggesting that this autoantibody may be involved in the pathogenesis as previously shown in animal models.[3,4] Although the patient numbers were small, they found that more Asians in the Nephrotic Syndrome Study Network (NEPTUNE) cohort had circulating nephrin autoantibodies during active disease and they had a short-relapse-free period.[2]

Herein, focusing on antinephrin-positive cases, we investigated clinicopathologic characteristics of antinephrin-positive primary podocytopathies in a cohort of Japanese older adult patients.

RESULTS

This is a single-center case series in Japan with 7 consecutive cases of adult-onset MCD presented between May 2020 to November 2021, which had paired kidney biopsy and serum samples. We measured antinephrin autoantibodies by enzyme-linked immunosorbent assay using previously published protocol[2] with some modification (Supplementary Methods, Supplementary Figure S1) and performed histopathological analysis using light, immunofluorescence, and electron microscopy. This study was approved by the Institutional Review Board of St. Marianna University School of Medicine (approval no.6223).

The median age of our cohort was 74 years (range 56–86), 71% male, median creatinine and median urine protein/creatinine ratio at diagnosis were 1.1 mg/dl (range 0.5–3.4) and 10.7 g/gCr (range 7.6–14.0 g/gCr), respectively. Their clinical course was summarized in the Supplemental Material (See Case descriptions in the Supplementary Material file. Supplemental Figure S2). Five out of 7 cases (71%) had circulating antinephrin autoantibodies at presentation (Table 1) and this finding correlated entirely with punctate podocyte IgG that colocalized with nephrin in the kidney biopsies of

Table 1. Clinical characteristics

Clinical characteristics	Antinephrin antibody-positive group					Antinephrin antibody-negative group	
Case	1	2	3	4	5	6	7
Age	56	75	74	76	86	65	63
Sex	Male	Male	Male	Male	Female	Male	Female
Comorbidities	Cerebral infarction	Bladder cancer	DM	None	None	None	Paralytic ileus
Serum antinephrin antibody at diagnosis (RU)	160	34	26	12	17	N/A	0
Punctate IgG on IF	+	+	+	+	+	-	-
Colocalization of nephrin and IgG on confocal microscopy	+	N/A	+	+	+	-	-
Baseline serum Cr (mg/dl)	0.7	0.6	1.2	0.8	0.8	0.8	0.4
Serum Cr at diagnosis (mg/dl)	3.4	0.8	3.0	2.0	1.1	0.9	0.5
Serum Alb at diagnosis (g/dl)	1.4	1.2	3.2	2.3	1.7	1.2	1.9
UPCR at diagnosis (g/gCr)	12.0	10.7	14.0	10.0	9.0	11.9	7.6
UPCR at "posttreatment" time point (g/gCr)	1.4 (PR)	0.26 (CR)	2.3 (PR)	0.4 (PR)	3.6 (PR)	0.07 (CR)	0.2 (CR)
Acute tubular injury	+	±	+	+	+	-	-
Total observation period (months)	40	35	91	31	26	36	27
Serum Cr at last follow-up (mg/dl)	1.4	0.9	1.1	1.2	0.8	0.8	0.4
Time to first recurrence (months)	18	11	15	19	-	-	-
Number of recurrences	2	1	4	1	-	-	-

Alb, albumin; CR, complete response; Cr, creatinine; DM, diabetes melitus; IF, immunofluorescent microscopy; N/A, not available; PR, partial response; RU, relative unit; UPCR, urine protein/creatinine ratio.

all antinephrin-positive patients (Supplemental Figures S3 and S4).

DISCUSSION

We highlight 3 findings in our cohort. First, while all 7 patients achieved complete response (CR; defined as urine protein/creatinine ratio <0.3 g/gCr)[5] after therapeutic interventions, those with antinephrin antibodies had a reduction in antinephrin antibody titer concordant with clinical response (Figure 1). The titer was markedly decreased in case 1 after the partial response (PR; defined as >50% reduction in proteinuria from disease onset, and/or urine protein/creatinine ratio 0.3–3.5 g/gCr),[5] and completely disappeared in the rest of the cases after either CR in case 2 or PR in case 3, 4, and 5. These results were consistent with the previous report showing antinephrin antibody titers were concordant with disease activity.[2]

Secondly, we observed a trend toward a shorter relapse-free period in the antinephrin antibody-positive group (median 18 months), compared to the negative group (no relapse), in keeping with the previous report ($P = 0.11$, log-rank test)[2] (Supplemental Figure S5). Four out of 5 patients with positive antinephrin antibodies experienced relapses during a follow-up period of 26 to 91 months. On the other hand, the 2 antinephrin antibody-negative cases had no recurrence during the 27 to 36 month follow-up period. Although the observation period for the positive cases was longer than that for the negative cases, the first recurrence in the positive cases occurred relatively

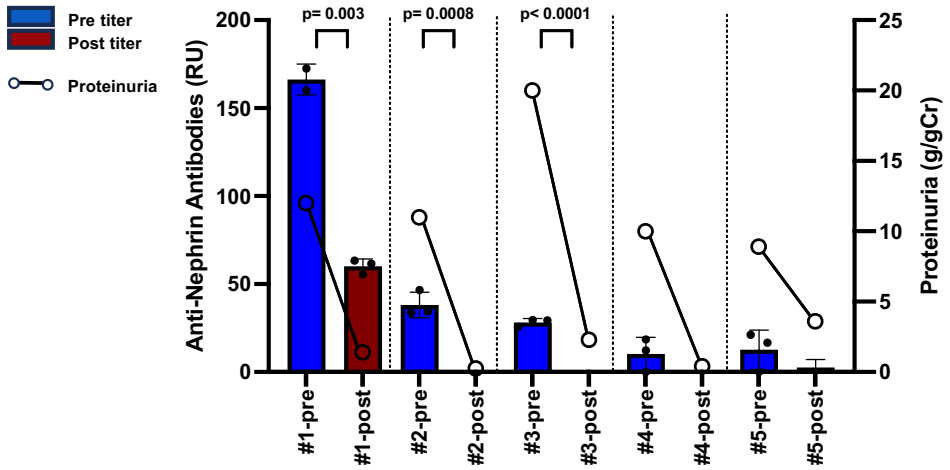

Figure 1. Antinephrin antibody titers before (blue) and after (red) therapeutic interventions. Proteinuria at the time of serum collection is shown by open circles. The nephrin autoantibody titers were compared by *t*-test. Cr, creatinine.

early (11–19 months after the first onset), suggesting that antinephrin antibody positivity may be associated with a higher risk of recurrence.

Third, we observed a higher frequency of acute kidney injury in the antinephrin positive group (60%) compared to the antinephrin negative group (0%). In all antinephrin-positive cases, acute tubular injury was observed in the kidney biopsies, but was not seen in antinephrin-negative cases (Table 1).

The presence of antinephrin autoantibody is emerging as a possible etiology of primary podocytopathies, including MCD.[2] While MCD is historically thought to have negative immunostaining for IgG and complement in kidney biopsies, the authors' careful observation led to the discovery of fine punctate podocyte staining of IgG that colocalized with nephrin. In our single-center cohort, we observed (1) a high proportion of antinephrin positive MCD in an exclusively Japanese adult cohort, (2) confirmed correlation of serum nephrin autoantibody titers with disease activity, and (3) noticed a shorter relapse-free period in the antinephrin antibody-positive group, consistent with the previous report.[2]

The clinical course of case 3 suggests that suppression of humoral immune response by B cell depletion therapies is effective in controlling disease activity of antinephrin antibody-positive MCD. In case 3, CD19-positive B cell proportions were suppressed to a very low level (0.4%) after initiation of rituximab treatment compared to that before the therapy (14.8%). The patient experienced the second and third relapse when CD19 counts became elevated to 1.5% and 3.9%, respectively, suggesting the possibility of relapse at the time of CD19-positive B cell recovery (Supplementary Figure S2C). Many studies in MCD have reported an association between relapse episodes and CD19-positive B cell recovery after rituximab treatment, and most cases showed a reappearance of B cells before relapse.[6] In addition, in case 3, MCD relapsed immediately after holding rituximab treatment to a mildly elevated CD19-positive B cell count of 1.2% and receiving a COVID-19 vaccination thereafter. It is possible that the vaccination in the setting of CD19-positive B cell recovery may have triggered undesirable autoantibody production (i.e., antinephrin autoantibody) and thereby contributed to another relapse of MCD. Close monitoring of CD19-positive B cell counts would be important to ensure suppression of humoral immune response when treating antinephrin positive MCD with B cell depletion therapies.

Acute kidney injury is common especially in older adults with MCD, with its incidence ranging 12% to 47%.[7] In our cohort, it is remarkable that up to 60% of patients with antinephrin positive MCD presented with acute kidney injury. Acute kidney injury associated with MCD is often attributed to nephrotoxic substances such as diuretics and nonsteroidal anti-inflammatory drugs, as well as decreased renal blood flow and volume depletion, but the exact mechanisms have yet to be fully understood. In our cases, morphologic evidence of acute tubular injury was present in all antinephrin antibody-positive cases but not in negative cases. The amount of proteinuria was similar in both groups and cannot explain the difference, and we need to await further investigation to draw firm conclusions on the mechanisms behind it.[8,9]

Our study has several limitations. First, the sample size was very small in this single-center cohort. Second, the follow-up period was relatively short, and longitudinal serum samples were not available to evaluate changes in antibody titers other than the pre- and post-treatment time points shown in this report.

In conclusion, our Japanese older adult cohort of patients with MCD revealed that the presence of circulating and glomerular antinephrin autoantibody is common, correlates with disease activity, and might be associated with a shorter relapse-free time. Future studies in larger cohorts with longer follow-up times are needed.

DISCLOSURE

All the authors declared no competing interests.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Supplementary Methods.

Case descriptions.

Figure S1. Positive and negative control values of antinephrin ELISA with modified protocol. Known antinephrin-positive samples (FR2, FR4, 21-242.1) were used as positive controls and healthy control samples (1-8) were used as negative controls. All negative control samples had 0 RU.

Figure S2. Clinical courses. (A–G) Clinical courses of antinephrin antibody-positive (AE) and negative (F–G) cases. Timepoints when biopsy and serum samples were taken are shown in the figures.

Figure S3. Confocal microscope images of kidney biopsy. Colocalization of IgG (green) and nephrin (red) staining was observed in antinephrin antibody-positive cases.

Figure S4. Immunofluorescent microscope images of kidney biopsy with IgG staining (green). Ant-nephrin positive samples had "fine punctate" staining on podocytes. An immunofluorescent imaging of membranous nephropathy case was included in inset, to highlight the coarse granular staining pattern, different from fine punctate staining in antinephrin positive cases.

Figure S5. Kaplan-Meier curve of the relapse-free time from diagnosis in the antinephrin antibody-positive group (red) and in the negative group (blue). Log-rank test $P = 0.11$.

STROBE Checklist.

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