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Association between anti-complement factor H antibodies and renal outcome in primary membranous nephropathy

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Abstract

Background: The complement factor H (CFH) regulates activation of the alternative complement pathway. Autoantibodies against CFH are involved in progressive renal dysfunction in cases with primary membranous nephropathy (MN). However, the prevalence and roles of anti-CFH antibodies in the clinical outcome of MN patients remain unclear.

Methods: We retrospectively investigated data of 36 Japanese patients with primary MN (23 men, 13 women; mean age: 64.5 [59-72] years) and 18 healthy normal controls (8 men, 10 women; mean age: 31 [27-38] years). Serum anti-CFH antibody titers were measured by enzyme-linked immunosorbent assay.

Results: Anti-CFH antibody titers were significantly higher in MN patients than in normal controls (4.69 [3.69-6.38] AU/mL vs. 0 [0-0] AU/mL, $p<0.001$). The patients were divided into groups: anti-CFH antibody positive group (n=28) and anti-CFH antibody negative group (n=8). No significant difference was observed in the remission rate of proteinuria and the incidence of 30% reduction of estimated glomerular filtration rate (eGFR) or 50% elevation of serum creatinine (Cr) levels between both groups. Anti-CFH antibody titer was selected as an independent unfavorable predictor of renal dysfunction by Cox proportional hazards analysis adjusted by age, gender, serum Cr levels, proteinuria (g/gCr), anti-CFH antibody titer, and immunosuppressive therapy (adjusted hazard ratio (HR) 1.344, 95% confidence intervals (CI) 1.038 to 1.741, p=0.025 for 30% reduction of eGFR; adjusted HR 1.930, 95% CI 1.108 to 3.363, p=0.020 for 50% elevation of serum Cr).

Conclusions: These data suggest that anti-CFH antibodies may be involved in the deterioration of renal function in primary MN.

Background

Membranous nephropathy (MN) is one of the major causes of nephrotic syndrome in adults. Histologically, reactive changes such as thickening of the glomerular basement membrane, spike formation, and stippling images are observed, and it is characterized by no associated proliferative changes. In adults, approximately 75% of cases are primary, the most common form, and approximately 25% are secondary to various causes such as tumors, infections, autoimmunity, and drugs. The course of MN is variable, and spontaneous remission is observed in about 30% of cases. However, treatment response to immunosuppressive drugs is poor and may progress to end-stage renal disease in approximately 40% of patients in Europe and North America.[1,2] In Japan, the renal survival rate of primary MN judged by a requirement for hemodialysis and/or end-stage disease (ESRD) with creatinine (Cr) levels ≥ 3.0 mg/dL was 60.5% of patients after 20 years. In this report, the poor prognostic factors were no immunosuppressive therapy including steroid monotherapy, male, age (60 years or older), renal dysfunction at onset (Cr ≥ 1.5 mg/dL), severe proteinuria at onset, focal glomerulosclerotic lesions, and advanced interstitial fibrosis.[3]

The pathogenic mechanism of MN has recently been elucidated from the aspect of autoantibodies. It is known that primary MN is induced by immune complexes composed with autoantigens expressed on the cell surface of glomerular epithelial cells and IgG4 predominant autoantibodies against them. As target autoantigens, thus far, an M-type phospholipase A_2 receptor (PLA2R), thrombospondin type-1 domain-containing 7A (THSD7A), and neural epidermal growth factor-like 1 protein have been reported, and account for approximately 70%, 1–5%, and 16% in primary MN, respectively.[4,5,6] The immune complex formed in the subepithelium locally induces complement activation and the formation of the membrane attack complex (C5b-9), the final product of the complement pathway. C5b-9 causes podocyte injury and may be involved in proteinuria in MN.[7,8] Complement factors C3 and C5b-9 are deposited along the glomerular tuft with the subepithelial immune complex.[9,10] Complement activation may be a major factor in the pathogenesis of MN, although the details of complement pathway involvement have not been clarified.

Recently, a case was reported in which renal dysfunction progressed, although anti-PLA2R antibody turned negative. In that case, the anti-complement factor H(CFH) antibody titer, an autoantibody against a complement regulatory factor, was observed to increase with renal dysfunction. As such, it was speculated that anti-CFH antibodies may have contributed to the deterioration of renal function in MN.[11] If an immune complex or complement activation is a damaging factor for disease, the complement regulatory factor is a host defense factor that leads to inactivation of activated complement. Complement activation of MN has been reported to involve alternative pathways,[12] and CFH is a major regulator of alternative pathways. CFH possesses three complement regulatory activities: the protein inhibits formation of the AP C3 convertase, accelerates the dissociation of this complex, and acts as a cofactor for the serine protease factor I.[13] Inhibition of CFH activity by anti-CFH antibodies on the cell surface of podocytes may cause an excessive enhancement of the alternative pathway and contribute to the progression of MN pathology. However, no clinicopathological studies have been conducted thus far to examine this hypothesis in a large cohort. In this study, we investigated whether anti-CFH antibodies are a risk factor for renal function deterioration or proteinuria persistence of MN in Japanese patients, including clinicopathological factors.

Methods

Patients

We retrospectively enrolled 36 Japanese MN patients who were admitted to Kanazawa Medical University Hospital. We followed these patients for at least 6 months (median [IQR], 69.0 [41.5–99.5] months). Diagnosis was confirmed in all patients by percutaneous needle renal biopsy. To detect secondary causes of MN, clinical workup, including detailed medical history and physical examination, serological analysis (tumor markers, autoantibodies, and infectious disease), and computer tomography (CT) scans, were conducted. The patients were treated non-randomly, depending on the judgment of the doctor in charge of each case. Serum anti-CFH antibodies were also measured in 18 healthy volunteers. The study

protocol was approved by the Clinical Study Ethics Review Board of Kanazawa Medical University. Verbal/written informed consent was obtained from all patients prior to study inclusion (Clinical Study Ethics Review Board of Kanazawa Medical University, Approval No. I425). This study was conducted according to the principles of the Declaration of Helsinki.

Measurement of serum anti CHF antibodies

Circulating antibodies to CFH were detected by using enzyme-linked immunosorbent assay (ELISA) plates coated with human complement CFH (Vidia Vestec, Czech Republic). Plates were incubated with the sera of patients and controls and revealed with horse radish peroxidase (HRP)-conjugated anti-human IgG antibody for total IgG detection according to the manufacturer's instructions. The measurable range of this ELISA kit was 3.9–250.0 AU/mL. Here, the data of < 3.9 AU/mL were judged arbitrary 0 AU/mL.

Laboratory And Pathological Examinations

We summarized the methods in short. Detailed methods were described in the previous paper[14].

Laboratory Examinations

Laboratory data such as serum creatinine and albumin levels, and urinary protein were evaluated in the standard methods. The estimated glomerular filtration rate (eGFR) was calculated by the 3-variable GFR-estimating equation for Japanese individuals ($194 \times SCr^{-1.094} \times age^{-0.287} \times 0.739$, if female).

Pathological Findings

Global sclerosis (%), interstitial fibrosis (score) and hyaline arteriolo-sclerosis (score) were examined using hematoxylin and eosin, periodic acid-Schiff (PAS), Masson trichrome, and periodic acid-silver methenamine (PAM) stainings.

Glomerular PLA2R, THSD7A and IgG subclasses (IgG 1, 2, 3, and 4) were assessed by indirect immunofluorescence in frozen sections using the primary antibodies listed in Supplementary Table S1. Immunofluorescence staining intensity was arbitrarily graded on a scale from 0 to 3 (0: negative, 1: weak staining, 2: moderate staining, and 3: strong staining) by at least two observers.

Ehrenreich and Churg classification for the ultrastructural staging of MN were assessed by electron microscopic study.

Endpoint And Definition

We measured the urine protein:creatinine ratio (uPCR) and evaluated the clinical status according to Japanese clinical categories as follows: the nephrotic state, that is, the presence of heavy proteinuria (> 3.5 g/gCr) and hypoalbuminemia (< 3.0 g/dL); incomplete remission type 2 (ICR2), that is, mean proteinuria of 1.0-3.5 g/gCr accompanied by an improvement of serum albumin levels (> 3.0 g/dL); incomplete remission type 1 (ICR1), that is, mean proteinuria of 0.3-1.0 g/gCr with normal serum albumin levels (> 3.0 g/dL); and complete remission (CR), that is, proteinuria of < 0.3 g/gCr with normal serum

albumin levels.[15] Renal function was evaluated by means of serum Cr and eGFR. Renal dysfunction was defined as 30% reduction of eGFR and 50% and 100% elevation of serum Cr from baseline.

Statistical analysis

Continuous measures and ranking scales were summarized using medians (25–75% IQR), whereas categorical measures were summarized using counts and percentages. The Mann-Whitney U-test was used to assess the differences between both groups. Fisher's exact tests and chi-square tests were utilized to compare proportions. The Kaplan-Meier life-table method and Cox proportional hazards analyses were used to evaluate predictors of clinical outcomes. A p value < 0.05 was regarded as significant.

Results

Characteristics Of Primary MN

Baseline laboratory data and anti-CFH antibody titers for primary MN and normal controls are shown in Table 1. The median ages of the 36 MN patients (23 males 63.9%) and 18 control patients (8 males 44.4%) were 64.5 years and 31.0 years, respectively. The anti-CFH antibody titer was positive in 28 of 36 cases in MN but was positive in only 1 of 18 cases in the normal control group. This was significantly higher in MN cases (MN, control group; 4.69 [3.69]-6.38] vs. 0.0[0.0–0.0], p < 0.001) (Fig. 1). Serum Cr levels were not different between both groups (0.82[0.69–0.89] vs 0.78[0.64–0.90], p = 0.378). The eGFR was significantly higher in the normal control group (68.6[54.5–79.3] vs. 84.9[75.8–91.9], p = 0.002), and the serum albumin level was also significantly higher in the normal control group (2.50[1.85–3.30] vs. 4.40 [4.20–4.70], p < 0.001). Total cholesterol levels were significantly higher in MN cases (251.5[215.0-313.5] vs. 194.0[181.0-212.0], p < 0.001). Median proteinuria level in MN cases was 4.59 g/gCr (2.01–7.33), and serum IgG was 890.5 mg/dL (556.5–1033.0). Among MN cases, 19 (52.8%) received immunosuppressive therapy, and 25 (69.4%) and 1 (2.8%) were positive for PLA2R and THSD7A, respectively. Additionally, ICR2 was observed in 35 cases (97.2%), ICR1 in 32 cases (88.9%), and CR in 23 cases (63.9%) during the observation period. Also, a 30% decrease in eGFR, 50% and 100% increases in serum Cr level were observed in 10 cases (27.8%), 8 cases (22.2%), and 3 cases (8.3%), respectively.

Table 1
Characteristics of patients with primary membranous nephropathy and normal controls

	Total (n = 54)	MN (n = 36)	Control (n = 18)	p value
Age	59.5 (38.0–67.0)	64.5 (59.0–72.0)	31 (27.0–38.0)	< 0.001
Gender, men/women	32/23	23/13	8/10	0.173
Anti CFH antibodies (AU/mL)	2.93 (1.84–4.91)	4.69 (3.69–6.38)	0 (0–0)	< 0.001
Serum creatinine (mg/dL)	0.81 (0.66–0.89)	0.82 (0.69–0.89)	0.78 (0.64–0.90)	0.378
eGFR (mL/min/1.73 m²)	74.3 (62.9–86.5)	68.6 (54.5–79.3)	84.9 (75.8–91.9)	0.002
Serum albumin (g/dL)	3.30 (2.20–4.20)	2.50 (1.85–3.30)	4.40 (4.20–4.70)	< 0.001
Total cholesterol (mg/dL)	222.5 (195.0-277.0)	251.5 (215.0-313.5)	194.0 (181.0-212.0)	< 0.001
Data are presented as median (IQR) unless otherwise indicated.				
MN, membranous nephropathy; CFH, complement factor H l; eGFR, estimated glomerular filtration rate				

Characteristics Of MN Patients With Or Without Anti-CFH Antibodies

MN cases were divided into two groups of positive and negative anti-CFH antibody titers, and the respective laboratory data and pathological findings are shown in Table 2. The median age of the 28 patients in the anti-CFH antibody positive group (20 men, 71.4%) and 8 patients in the anti-CFH negative group (3 men, 37.5%) were both 64.5 years. The median value of the anti-CFH antibody positive group was 5.31 (4.29–7.05). In the clinical data, only the total cholesterol level was significantly different, and was significantly higher in the anti-CFH antibody negative group (234.5[212.5-274.5] vs. 313.5[267.5–405.0], p = 0.006). In contrast, serum Cr level (0.82[0.72–0.90] vs. 0.82[0.63–0.88], p = 0.518), eGFR(68.1[57.5–80.3] vs. 72.6[52.5–74.4], p = 0.732), serum albumin level (2.55[1.90–3.50] vs. 2.20[1.75–2.55], p = 0.114), proteinuria volume (4.13[1.49–6.37] vs. 6.68[4.10–14.4], p = 0.080), and IgG (934.0[587.0-1055.5] vs. 691.5 [446.5-879.5], p = 0.057) did not show any significant difference between the two groups. In the anti-CFH antibody positive group and negative group, 14 cases (50.0%) and 5 cases (62.5%) received immunosuppressive therapy, 14 cases (50.0%) and 3 cases (37.5%) received

supportive therapy, 18 cases (64.3%) and 7 cases (87.5%) were PLA2R-positive, and 1 case (3.9%) and 0 case (0%) were THSD7A positive, respectively.

Table 2
Characteristics of primary membranous nephropathy patients with or without anti-CFH antibodies.

	Anti-CFH antibodies positive (n = 28)	Anti-CFH antibodies negative (n = 8)	p value
Age	64.5 (58.5–72.0)	64.5 (62.5–70.5)	0.68
Gender, men/women	20/8	3/5	0.107
Serum creatinine (mg/dL)	0.82 (0.72–0.90)	0.82 (0.63–0.88)	0.518
eGFR (mL/min/1.73m²)	68.1 (57.5–80.3)	72.6 (52.5–74.4)	0.732
Serum albumin (g/dL)	2.55 (1.90–3.50)	2.20 (1.75–2.55)	0.114
Total cholesterol (mg/dL)	234.5 (212.5-274.5)	313.5 (267.5–405.0)	0.006
Serum IgG (mg/dL)	934.0 (587.0-1055.5)	691.5 (446.5-879.5)	0.057
Urinary protein* (g/gCr)	4.13 (1.49–6.37)	6.68 (4.10–14.4)	0.080
Anti CFH antibodies (AU/mL)	5.31 (4.29–7.05)	0	< 0.001
Immunosuppressive therapy, n (%)	14 (50%)	5 (62.5%)	0.695
Supportive therapy, n (%)	14 (50%)	3 (37.5%)	0.695
Pathological findings			
Immunofluorescence findings			
PLA2R-positive, n (%)	18 (64.3%)	7 (87.5%)	0.388
THSD7A-positive, n (%)	1 (3.6%)	0 (0%)	1.000
IgG1 score	2.0 (1.0–3.0)	1.5 (1.0-2.5)	0.678
IgG2 score	1.0 (0.0–1.0)	0.5 (0.0–1.0)	0.680
IgG3 score	1.0 (0.0–3.0)	1.0 (0.0–2.0)	0.574
IgG4 score	2.0 (0.5-3.0)	2.0 (1.0–3.0)	1.000
Light microscopic findings			
Global sclerosis (%)	7.75 (0.0-18.80)	10.95 (3.25–21.35)	0.271
Interstitial fibrosis score	1.0 (0.0–1.0)	1.0 (1.0–1.0)	0.707

Data are presented as median (IQR) unless otherwise indicated. *: urinary protein to creatinine ratio. CFH, complement factor H; eGFR, estimated glomerular filtration rate; IgG, immunoglobulin G; Cr, creatinine; PLA2R, phospholipase A 2 receptor; THSD7A, Thrombospondin type-1 domain-containing 7A

	Anti-CFH antibodies positive (n = 28)	Anti-CFH antibodies negative (n = 8)	p value
Hyaline arteriolosclerosis score	1.0 (0.0–3.0)	1.0 (0.0-2.5)	0.767
Electron microscopic findings			
Ehrenreich-Churg stage	2.0 (1.0–3.0)	2.0 (1.25–2.75)	0.544

Data are presented as median (IQR) unless otherwise indicated. *: urinary protein to creatinine ratio. CFH, complement factor H; eGFR, estimated glomerular filtration rate; IgG, immunoglobulin G; Cr, creatinine; PLA2R, phospholipase A 2 receptor; THSD7A, Thrombospondin type-1 domain-containing 7A

The following pathological data did not show any significant difference between both groups: the score indicating the staining degree of each IgG subclass of IgG1, IgG2, IgG3, and IgG4 (2.0[1.0–3.0] vs. 1.5[1.0-2.5], p = 0.678), (1.0[0–1.0] vs. 0.5[0–1.0], p = 0.680), (1.0[0–3.0] vs. 1.0[0–2.0], p = 0.574), (2.0[0.5-3.0] vs. 2.0[1.0–3.0], p = 1.000), interstitial fibrosis score (1.0[0–1.0] vs. 1.0 [1.0–1.0], p = 0.707), and arteriosclerosis score (1.0[0–3.0] vs. 1.0[0-2.5], p = 0.767). There was also no significant difference between both groups in the stages of Ehrenreich-Churg classification (2.0[1.0–3.0] vs. 2.0[1.25–2.75], p = 0.544).

Clinical Outcomes Of MN

The remission of proteinuria and the occurrence of renal dysfunction between the positive and negative anti-CFH antibody groups in MN cases were examined by the Kaplan-Meier method. Achievement of ICR2, ICR1 (Fig. 2A), and CR (Fig. 2B), eGFR decrease of 30% (Fig. 3A), serum Cr increase of 50% (Fig. 3B), and 100% were not significantly different (ICR2; p = 0.563, ICR1; p = 0.728, CR; p = 0.686, eGFR 30% decrease; p = 0.961, serum Cr 50% increase; p = 0.662, serum Cr 100% increase; p = 0.316). However, univariate Cox proportional hazard analysis revealed that the anti-CFH antibody titer was an unfavorable predictor of serum Cr level increase of 50% (hazard ratio (HR) 1.294, 95% confidence intervals (CI) 1.039 to 1.613, p = 0.022, Table 3). Moreover, a multivariate Cox proportional hazard analysis adjusted for baseline age, sex, serum Cr level, proteinuria (uPCR), and the presence or absence of immunosuppressive therapy showed that anti-CFH antibody titer was an independent unfavorable predictor for a 30% decrease in eGFR (adjusted HR 1.344, 95% CI 1.038 to 1.741, p = 0.025, Table 3) and a 50% increase in serum Cr (adjusted HR 1.930, 95% CI 1.108 to 3.363, p = 0.020, Table 4).

Table 3
Predictors of eGFR 30% reduction in patients with primary membranous nephropathy

	Univariate		Multivariate	
	HR [95%CI]	P-value	HR [95%CI]	P-value
Age	1.001 [0.908–1.105]	0.975	0.987 [0.884–1.102]	0.820
Men (versus women)	0.779 [0.193–3.145]	0.726	0.696 [0.104–4.642]	0.708
Anti-CFH antibody titre	1.184 [0.965–1.453]	0.105	1.344 [1.038–1.741]	0.025
Serum Cr (per 0.1 mg/dL)	2.197 [0.589–8.197]	0.241	1.677 [0.218–12.92]	0.620
uPCR (per 1.0 g/gCr)	1.100 [0.968–1.251]	0.145	1.184 [0.967–1.449]	0.102
Immunosuppressive treatment (versus supportive)	1.472 [0.394-5.500]	0.565	1.313 [0.246–7.01]	0.750
eGFR, estimated glomerular filtration rate; HR, hazard ratio; CI, confidence interval.				
Data are the HR, 95% CI and p-value from Cox proportional hazard regression analyses.				
CFH, complement factor H; Cr, creatinine, uPCR, urinary protein to creatinine ratio.				

Table 4
Predictors of serum creatinine 50% elevation in patients with primary membranous nephropathy

	Univariate		Multivariate	
	HR [95%CI]	P-value	HR [95%CI]	p-value
Age	1.000 [0.900-1.111]	0.997	1.055 [0.918–1.212]	0.450
Men (versus women)	0.571 [0.127–2.564]	0.464	0.272 [0.032–2.349]	0.237
Anti-CFH antibody titre	1.294 [1.038–1.612]	0.022	1.930 [1.108–3.363]	0.020
Serum Cr (per 0.1 mg/dL)	2.266 [0.617–8.322]	0.218	0.751 [0.045–12.47]	0.842
uPCR (per 1.0 g/gCr)	1.120 [0.976–1.285]	0.108	1.138 [0.868–1.493]	0.350
Immunosuppressive treatment (versus supportive)	2.854 [0.553–14.74]	0.211	13.0 [0.287–586.1]	0.188
HR, hazard ratio; CI, confidence interval.				
Data are the HR, 95% CI and p-value from Cox proportional hazard regression analyses.				
CFH, complement factor H; Cr, creatinine, uPCR, urinary protein to creatinine ratio.				

Discussion

This is the first cohort study to examine the clinical significance of anti-CFH antibodies in Japanese patients with primary MN, and we obtained three new findings. First, the anti-CFH antibody positive rate was significantly higher in patients with MN than control patients. Second, elevated anti-CFH antibody titers were a risk factor for renal dysfunction. Third, no correlation was observed between the amount of anti-CFH antibody titer and the amount of urinary protein.

Here, it was revealed that primary MN cases had a higher anti-CFH antibody positive rate and a significantly higher antibody titer than the normal control group. As for the involvement of anti-CFH antibodies in renal diseases, atypical hemolytic-uremic syndrome (aHUS) is well known. Anti-CFH antibodies have been found in approximately 10–20% of aHUS cases. Anti-CFH antibody-associated aHUS cases have a poor prognosis and are prone to recurrence, similar to un-associated aHUS cases, with approximately 50% reportedly progressing to ESRD.[16,17,18] Although the appearance of anti-CFH antibodies in aHUS is reportedly mostly associated with genetic abnormalities, some are confirmed not to have genetic abnormalities, and have been observed to emerge due to an acquired factor.[19] MN is predicted to emerge from an acquired factor depending on the age of onset, but as with other autoimmune diseases, the mechanism of development is unclear. Moreover, it was unclear in this study

whether the appearance of anti-CFH antibodies occurred after the onset of MN or were retained before the onset of MN. Since one positive case was observed even in the normal control group, it is possible that there are cases with anti-CFH antibodies as a background pathological condition, that may be involved in the onset of MN itself. In order to clarify these, a large-scale prospective study, including normal controls, is needed.

This study revealed that anti-CFH antibody titer was an independent unfavorable predictor of renal dysfunction in MN cases. Thus far, the only report on the association between MN and anti-CFH antibodies is the case report wherein the progression of renal dysfunction was observed with the increase in anti-CFH antibody titre.[11] Since an effect on renal dysfunction was observed, it is speculated that the anti-CFH antibodies had a stronger effect on glomerular and/or interstitial injuries. It was also reported from a study on aHUS that C5b-9 injures glomerular endothelial cells.[20] As such, the renal injury caused by anti-CFH antibodies in this study may have involved microvascular injury, such as aHUS. In mouse models of nephrotic syndrome, IgA nephropathy, and lupus nephritis, C5b-9 deposition has been shown to be involved in renal tubulo-interstitial injury.[21,22,23] Here, no difference was found in the tubulo-interstitial lesions and glomerular function at baseline between the anti-CFH antibody positive and negative patients. Further study with a larger scale is needed to elucidate the mechanism of renal injury in primary MN caused by anti-CFH antibodies.

In this study, anti-CFH antibody titer was not associated with the levels of proteinuria and clinical remission in primary MN cases. However, it was well established that C5b-9 causes podocyte injury and is involved in proteinuria in MN. Furthermore, glomerular deposition of CFB was reported in the histological findings of MN,[9] and MN was also found in cases of C4 deficiency,[24] suggesting that an alternative pathway is involved in MN complement activation. CFH, a major regulator of the alternative pathway, is a single-chain liquid protein with a molecular weight of 150 kDa and is composed of 20 short consensus repeats (SCR). The binding site to C3b is the C-terminal domain (SCR19-20).[25] It has been reported that anti-CFH antibodies suppress the regulatory function by binding to SCR15-20, a part of the CFH binding site.[26] These accumulated findings lead us to speculate that CFH may have an impact on proteinuria. However, our data did not support this speculation. A potential explanation could be that low titer anti-CFH antibodies could not sufficiently reach the podocyte condition in vivo. Meanwhile, in this study, anti-CFH antibody titer was not associated with the levels of proteinuria and clinical remission in primary MN cases. Hence, it is likely that the low titer of anti-CFH antibodies may not be involved in podocyte injury.

The antibody titers obtained in this study were low compared to previous case reports on aHUS.[11] In the case of aHUS, the cut-off value for anti-CFH antibody titer is reported to be 100 AU/mL.[17] aHUS with a genetic abnormality or anti-CFH antibodies causes abnormal activation of an alternative pathway triggered by infection and inflammation, resulting in thrombocytopenia and renal injury due to thrombosis and hemolysis in microvessels. As the antibody titer in MN reported in this study was not as high as that of aHUS, the clinical findings on blood vessels may be milder than that of aHUS. However, the circulating anti-CFH antibody titer was negatively correlated with proteinuria and was elevated after remission in

some cases (data not shown). Anti-CFH antibodies may result in loss of urine, because two-thirds of the cases at baseline had nephrotic syndrome and anti-CFH antibody negative cases showed lower serum IgG levels (additional file 1, p = 0.057). Thus, a long-term observation is required to define the exact relation between circulating anti-CFH antibodies and proteinuria. It is necessary for future studies to examine with greater detail the effect of different antibody titers on vascular endothelium, glomerular, and tubulo-interstitial injuries.

The notable point of this study was that the non-invasive and simple test of anti-CFH antibody titer was able to predict poor prognosis of MN renal function at an early stage. If the baseline anti-CFH antibody titer can be used to determine the risk of developing end-stage renal failure, an appropriate treatment method can be promptly selected.

There are two main limitations in this study. First, because this was a single-center study with a small sample size, the generalizability of the results is limited. Secondly, this study showed an epidemiological causal relationship between anti-CFH antibody titer and prognosis of renal function, although the mechanism by which anti-CFH antibodies causes renal dysfunction is still unknown. Therefore, it is necessary to carry out a future follow-up study that will target a large number of MN patients at multiple centers, and to conduct basic research on the mechanism of anti-CFH antibodies causing renal dysfunction.

Hence, this study suggested that anti-CFH antibody titer was an independent unfavorable predictor of renal dysfunction in primary MN patients. It signifies that in the future, anti-CFH antibody titer could be a biomarker of renal prognosis in primary MN.

List Of Abbreviations

aHUS, atypical hemolytic-uremic syndrome

C5b-9, membrane attack complex

CFH, complement factor H

CI, confidence intervals

CT, computed tomography

eGFR, glomerular filtration rate

ESRD, end-stage disease

HR, hazard ratio

ICR1, incomplete remission type 1

ICR2, incomplete remission type 2

MN, membranous nephropathy

PAM, periodic acid-silver methenamine

PAS, periodic acid-Schiff

PLA2R, phospholipase A 2 receptor

SCR, short consensus repeats

Cr, serum creatinine

uPCR, urinary protein to creatinine ratio

Declarations

Ethics approval and consent to participate

Verbal/written informed consent was obtained from all patients (Clinical Study Ethics Review Board of Kanazawa Medical University, approval numbers I425). This study was conducted according to the principles of the Declaration of Helsinki and Istanbul.

Consent for publication

Not applicable

Availability of data and materials

The dataset supporting the conclusions of this article is included within the article and the attached data sheet.

Competing Interest

The authors declare no conflict of interest.

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Author Contributions

YK wrote the manuscript, interpreted clinical data, and performed ELISA experiments. NH designed the study and directed the immunohistochemistry study. K. Fujimoto supervised the statistical analysis and helped collect clinical data. HA performed kidney biopsies and helped in collecting clinical data. K. Furuich interpreted histopathology and edited the manuscript. HY designed the study and supervised the manuscript editing as a general adviser and planner of this study. The manuscript was drafted and written by YK, with input as appropriate from the rest of the investigators.

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Figures

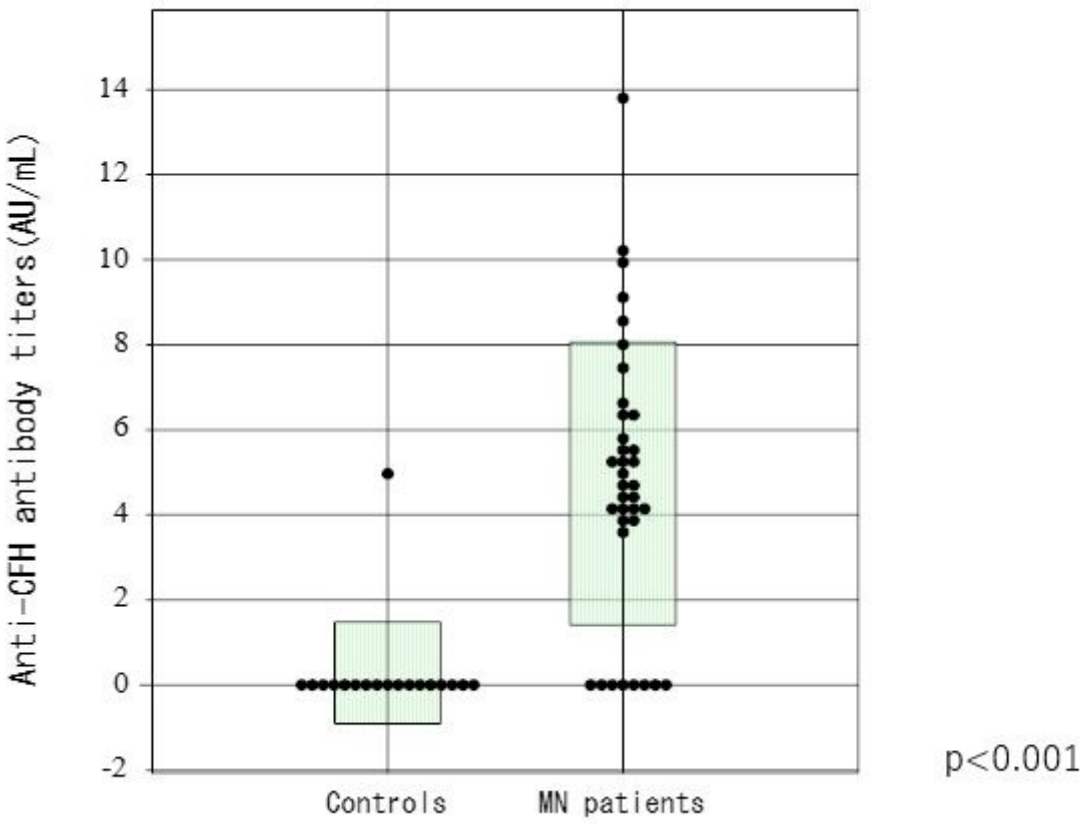

Figure 1

Comparison of anti-CFH antibody titres in patients with membranous nephropathy and normal controls. There is a significant difference in serum anti-CFH antibody titres between patients with primary membranous nephropathy and healthy normal controls (4.69 [3.69-6.38] vs. 0 [0-0], p<0.001 by Mann–Whitney U-tests). CFH, complement factor H l.

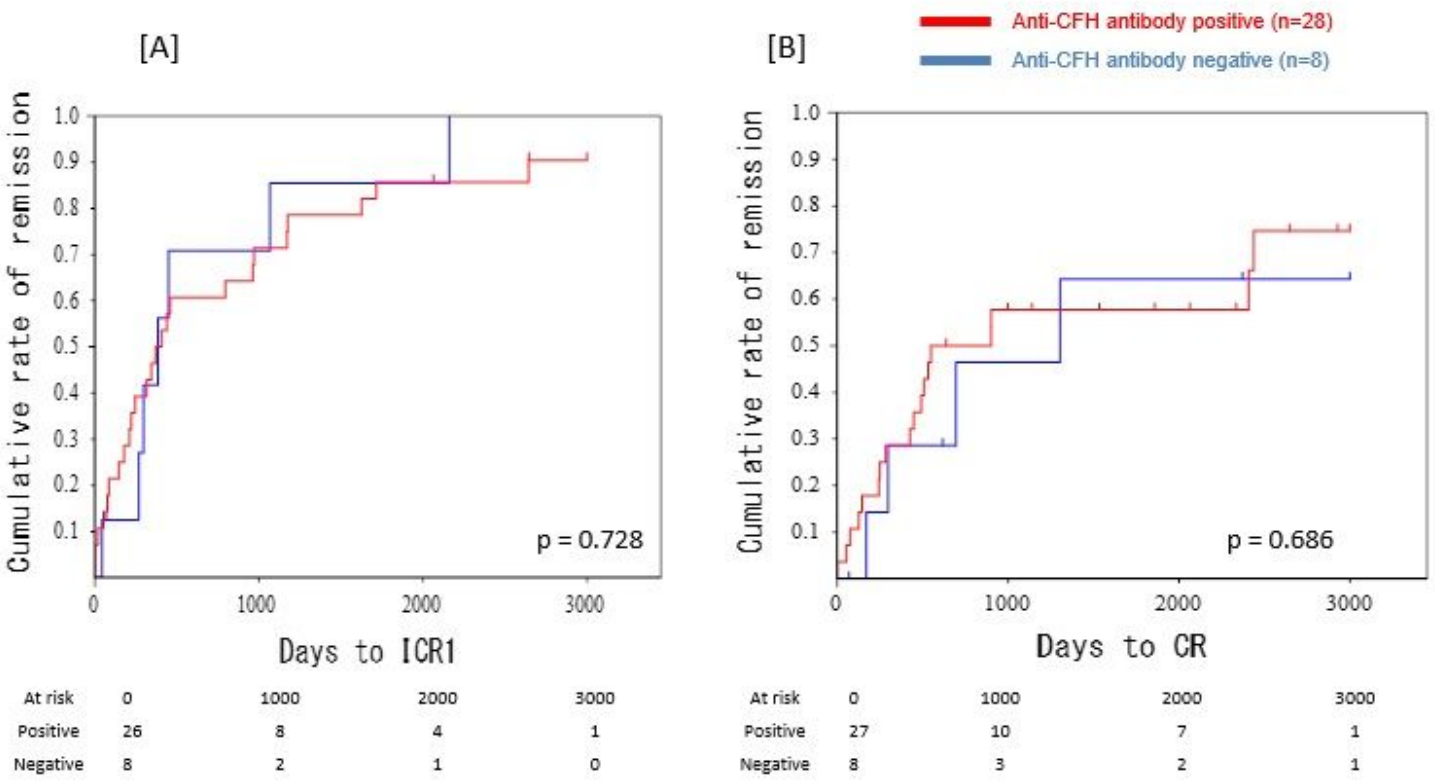

Figure 2

Remission of proteinuria in patients with membranous nephropathy with or without serum anti-CFH antibodies. The patients with anti-CFH antibody positive membranous nephropathy show similar outcomes in the achievement of ICR1 (uPCR <1.0) [A] and ICR2 (uPCR <0.3) [B] to those without anti-CFH antibodies. Differences between the two groups were compared using Kaplan-Meier curves with the log-rank test. CFH, complement factor H l; ICR1, incomplete remission type 1 ; ICR2, incomplete remission type 2; uPCR, urine protein: creatinine ratio.

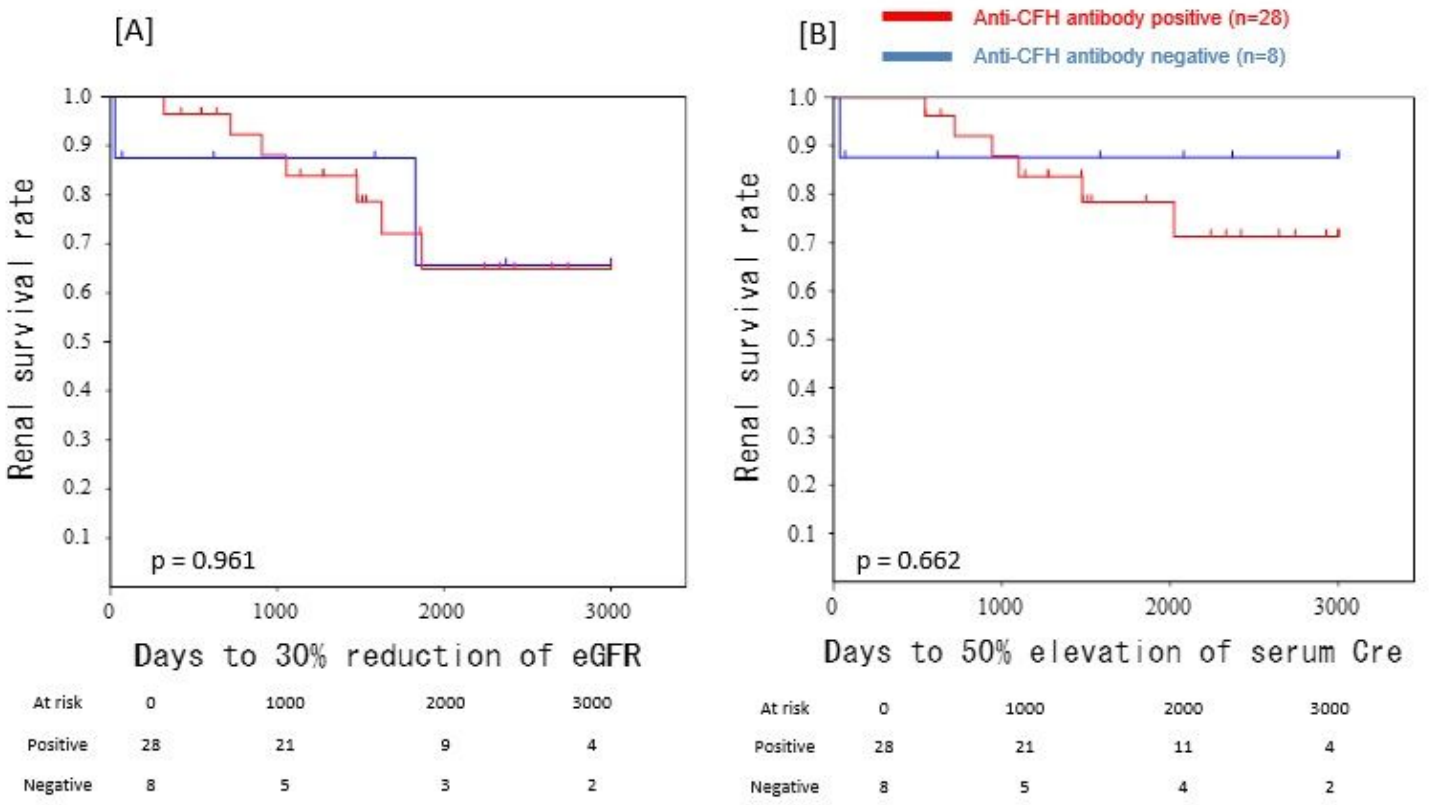

Figure 3

Incidence of renal dysfunction in membranous nephropathy patients with or without serum anti-CFH antibodies. The patients with anti-CFH antibody positive membranous nephropathy show similar outcomes in renal dysfunction with 30% reduction of eGFR from baseline [A] and 50% elevation of serum creatinine levels [B] compared with those without anti-CFH antibodies. Differences between the two groups are compared using Kaplan-Meier curves with the log-rank test. CFH, complement factor H l; eGFR, estimated glomerular filtration rate.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Dataofprimarymembranousnephropathy36casespdf.pdf
- SupplementaryTableS1.docx