Clinical and prognostic significance of glomerular C1q deposits in primary MN

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ABSTRACT

Background: Although complement activation is believed to be important in mediating PMN, the pathways involved and clinical consequences remain controversial. Many cases of idiopathic or primary membranous nephropathy (PMN) present with subepithelial C1q deposits along with IgG and C3 on glomerular capillary walls but without deposits of IgA or IgM ("full house") by immunofluorescence or any causes of secondary MN. We sought to define the clinical and pathological significance of these C1q deposits in PMN by comparing a variety of clinical parameters, outcomes and other serum and urine factors in patients with and without significant glomerular C1q deposits.

Methods: Two-hundred eighty-eight patients with biopsy-proven PMN were enrolled. We compared the clinical and pathological features, treatment responses and kidney outcomes, between patients with and without C1q deposition. Circulating anti-PLA2R antibodies and complement components in plasma and urine were detected by ELISA.

Results: Glomerular C1q deposition was detected on capillary walls by immunofluorescence in 66/288 (22.9%) patients. C1q-positive patients presented with lower concentrations of serum IgG (5.3 ± 3.1 vs. 6.6 ± 3.5 g/l, p = 0.008), a higher frequency of IgA (37.9% vs. 15.8%, p < 0.001), IgM (48.5% vs. 31.5%, p = 0.011) and C3c (100% vs. 88.3%, p = 0.004) deposits in glomeruli and more stage III of MN (24.2% vs. 11.7%, p < 0.001) by pathologic criteria. Other features, including gender, age, anti-PLA2R antibody positivity and concentrations, proteinuria, albumin and serum creatinine, were not different between the patients with and without C1q deposition (p > 0.05). The IgG subclasses of anti-PLA2R antibodies in circulation or in glomeruli showed no difference (p > 0.05). C1q deposition, and C1q concentrations in circulation and urine had no apparent effect on the treatment responses or kidney outcomes (p > 0.05).

Conclusion: The classical pathway of complement is activated in some patients with PMN, but may not play an essential role in mediating the kidney injury seen in this disease.

1. Introduction

Primary membranous nephropathy (PMN) is an autoimmune-mediated glomerular disease that is one of the most common causes of nephrotic syndrome in adults and may progress to end-stage kidney disease (ESKD) in the long term. The kidney histology is characterized by thickening of glomerular basement membrane with spike formation, granular deposits of IgG and complement C3, and subepithelial electron-dense deposits [1]. Recent studies have shown that circulating IgG antibodies against M-type phospholipase A2 receptor (PLA2R) are detectable in both serum and glomeriular deposits in about 70% of patients with PMN [2]. These findings replicate earlier observations in the Heymann nephritis models of PMN in rats in which subepithelial deposits of IgG and C3 reflect in situ immune deposit formation involving an IgG anti-podocyte antibody and proteinuria is mediated by complement through the C5b-9 membrane attack complex [3–5]. However, studies to date in PMN have not clearly defined whether complement activation is pathogenic in the human disease or which pathway of activation might be involved [6].

In man IgG4 is the major subclass of IgG in anti-PLA2R deposits in glomeruli [7]. However, IgG4 is unable to activate the complement system efficiently through the classical pathway [8]. In contrast, IgG1

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and IgG3 are commonly detected in the immune-complex deposits of secondary MN [1] in which the complement system is intensively activated, usually through the classical pathway [9]. Because C1q deposition along glomerular capillary walls is an uncommon finding in PMN it is often considered as a marker of secondary MN [10].

Although the finding of a full house pattern by immunofluorescence (i.e. the presence of IgG, IgM, IgA, C3, and C1q) in glomeruli is very common in secondary MN, particularly lupus membranous nephropathy [9], we see many cases of PMN that present with C1q deposition together with only IgG and C3 granular deposits along glomerular capillary walls [9, 11, 12]. Although PMN is diagnosed in these patients and the majority of whom are PLA2R-associated, the clinical and pathological significance of C1q deposition in glomeruli is unclear. In addition, the role of circulating C1q in PMN patients still needs further clarification.

2. Materials and methods

2.1. Patients and samples

Two-hundred eighty-eight patients with kidney-biopsy proven PMN were enrolled and followed up in Peking University First Hospital from 2006 to 2013. Patients with secondary MN (including hepatitis B virus infection, systemic lupus erythematosus, malignancy, medication, and heavy metal poisoning) were excluded by clinical criteria as well as complete serologic evaluation including the detection of ANA, anti-dsDNA antibodies, Sm antibodies, HBV-DNA copies, etc., and the kidney pathologic features including the "full-house" pattern of immunofluorescence, HBs and HBc antigens deposits in glomeruli, etc. Medical records and laboratory data were collected at the time of kidney biopsy and during follow up. The research was in compliance of the Declaration of Helsinki and approved by the ethics committee of Peking University First Hospital. Informed consent was obtained for the use of tissue and blood for medical and research purposes.

Plasma and urine samples were available from 106 patients. The clinical and pathological features were comparable between the patients with and without plasma and urine samples. The samples were collected on the day of kidney biopsy before immunosuppressive treatments were started. The plasma and urine samples from 25 age and gender matched healthy donors were collected as normal controls. The samples were stored in aliquots at −80 °C until use.

2.2. Kidney biopsies

Kidney biopsy was performed at the time of diagnosis for all patients. The specimens were evaluated with direct immunofluorescence, light and electron microscopy [13]. Immunofluorescence staining (all antibodies from Dako) intensity was semi-quantitatively scored from 0 to 3+ (0, negative; 1+, weak staining; 2+, moderate staining; 3+, strong staining). In evaluating glomerular IF staining, only deposits present along glomerular capillary walls were scored. Glomerular MN lesions studied by EM were graded into four stages according to Ehrenreich and Churg's classification criteria [14].

2.3. Quantification of C1q

A sandwich enzyme-linked immunosorbent assay (ELISA) technique was used for the detection of C1q in plasma and urine as described previously [15]. In brief, microassay plates (Costar, Mankato, MN, USA) were coated by murine monoclonal antibodies binding specifically to human C1q (Dako, CA, USA) 1:5000 overnight at 4 °C. After washing, the coated wells were blocked with 1% BSA for 1 h at 37 °C. Plasma (1:2000) and urine (1:2) samples were added and incubated at 37 °C for 1 h. After washing, horseradish peroxidase conjugated goat anti-human C1q antibodies (Abcam) 1:500 were added and incubated for 1 h at room temperature. Chromogenic substrate (Thermo Fisher Scientific)

1:1 was added and after 30 min the color development was stopped with 1.0 M H_2SO_4. The results were recorded as the net optical absorbance at 405/570 nm (Bio-Rad 550). The concentrations of urinary C1q were corrected for the concentrations of urine creatinine.

2.4. Quantification of other complement components

Commercial ELISA kits (Quidel) were used for the detection of MBL, C4d, Bb, properdin, C3a, C5a and soluble C5b-9 (SC5b-9) in plasma and urine. All complement proteins were assayed following the manufacturer's instructions. The protocol was a five-step procedure: 1) Microassay plates were pre-coated with murine monoclonal antibodies binding specifically to the complement components. 2) Plasma and urine samples were added according to the optimal dilution, incubation time and room temperature based on the manufacturer's instructions. 3) Horseradish peroxidase conjugated antibodies were added and bound to the complement components. 4) Chromogenic substrate was added to determine the concentration of complement components. 5) Results were recorded as the net optical absorbance (Bio-Rad 550, Tokyo, Japan). The concentrations of urinary complement components were corrected by the concentrations of urine creatinine.

2.5. Detection of circulating anti-PLA2R antibodies

Circulating anti-PLA2R antibodies were detected by using commercial ELISA kits (EUROIMMUN AG, Lübeck, Germany). In brief, patients' plasma was diluted in PBST to 1:100, then added into the reaction area on microplates and incubated at room temperature for 30 min. After washing, the microplates were incubated with enzyme-conjugated secondary antibodies at room temperature for 30 min. and the results were recorded as the net optical absorbance at 450 nm (Bio-Rad 550, Tokyo, Japan). Antibody positivity was defined as concentrations > 20 U/ml [16]. Anti-PLA2R IgG subclasses were detected as described above, with HRP-conjugated anti-human IgG1, IgG2, IgG3, and IgG4 antibodies (SouthernBiotech) diluted 1:1000 and incubated at 37 °C for 60 min.

2.6. Responses to treatment and kidney outcomes

The use of steroids and immunosuppressive agents and the evaluation for treatment responses at our center were in compliance with the 2012 KDIGO (Kidney Disease: Improving Global Outcomes) guidelin7e for glomerulonephritis [17].

To evaluate the kidney outcomes, the primary endpoint was ESKD, and the secondary endpoint was kidney dysfunction, defined as eGFR falling by > 30% from the baseline at the time of kidney biopsy and to < 60 ml/min/1.73m^2.

2.7. Statistical analysis

Student's *t*-test (for normally distributed data) or a nonparametric test (for non-normally distributed data) were used to compare the differences in quantitative parameters between groups. The relations were analyzed by Pearson's correlation test (between two normally distributed variables) or Spearman's correlation test (between two non-normally distributed variables). Risk factors for renal outcomes and treatment responses were analyzed using the Cox regression model and a logistic regression model. Results were expressed as hazard ratios (HR) and 95% confidence interval (CI). All statistical analyses were two-tailed and p < 0.05 was considered significant. Analysis was performed with the SPSS statistical software package, ver 19.0.