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Original article

Complement deposition on renal histopathology of patients with diabetic nephropathy

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ABSTRACT

Aims. – As the potential role of the complement system in diabetic nephropathy (DN) is increasingly reported, this study aimed to investigate C1q and C3c deposition as seen on renal histopathology, as well as its association with clinical and pathological parameters, in DN patients.

Methods. – Renal biopsy specimens from 161 DN patients were investigated using direct immunofluorescence, light, and electron microscopy. For direct immunofluorescence, staining for C1q and C3c on fresh-frozen renal tissue was performed immediately after biopsy. Complement deposition was defined as the presence of C1q or C3c of at least 1 + on a 0–4 + Scale. The association between complement deposition and clinicopathological data was also analyzed.

Results. – On direct immunofluorescence microscopy, C1q and C3c were detected in specimens from 44/161 (27.3%) and 89/161 (55.3%) patients, respectively. Regarding clinical data, patients with C1q deposition had a significantly higher level of urinary protein (7.25 ± 4.20 g/24 h vs. 4.97 ± 3.76 g/24 h; $P < 0.01$) and significantly lower estimated glomerular filtration rate (eGFR; 34.16 ± 25.21 mL/min/1.73 m^2 vs. 51.17 ± 31.56 mL/min/1.73 m^2, respectively; $P < 0.01$), whereas patients with vs. without C3c deposition had a significantly lower eGFR (40.09 ± 27.97 mL/min/1.73 m^2 vs. 54.48 ± 32.49 mL/min/1.73 m^2, respectively; $P < 0.01$). On renal histopathology, patients with C1q deposition had significantly higher Scores for interstitial fibrosis and tubular atrophy (IFTA), interstitial inflammation and vascular lesions ($P < 0.01$, $P < 0.05$ and $P < 0.05$, respectively), whereas patients with C3c deposition had significantly higher IFTA Scores and proportions of global sclerosis ($P < 0.01$ and $P < 0.01$, respectively).

Conclusion. – Complement deposition of C1q and C3c on renal histopathology is associated with more severe kidney damage in patients with DN.

Introduction

Diabetic nephropathy (DN), a serious complication of diabetes mellitus (DM), is the leading cause of end-stage kidney disease worldwide. In mainland China, it was estimated that 109.6 million adults have DM [1], while chronic kidney disease (CKD) related to diabetes has become more common than glomerulonephritis in China in recent years [2]. Although genetic susceptibility [3,4], hyperglycaemia [5,6] and hypertension [7] are thought to play major roles in the initiation and progression of DN, its pathogenesis remains largely unclear. However, in recent decades, immune-mediated inflammation has been increasingly recognized as having an important role in the development of DN [8–10].

As a moderator of both the innate and adaptive immune systems, the complement cascade is a host defense system against diseases such as infections [11], and can be activated through three major pathways: the classic; the alternative; and the mannose-binding lectin (MBL) pathway. These all lead to the production of C3 convertase and, eventually, the generation of membrane attack complex (MAC), which has biological and pathological functions. Recently, the potential role of the complement system in DN has been increasingly reported [12–17]. Therefore, it is of interest to further investigate the clinical and pathological significance of complement deposition as seen on renal histopathology in DN patients.

With renal histological examinations at our group's clinical practice, C1q and C3c staining are routinely performed. As a key

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component of the classic pathway, C1q combines with more than two Fc segments and activates downstream [18]. C3 is predominantly produced in the liver and is the central component of the entire complement system, activated by all three pathways and resulting in cleavage of C3 into C3a, C3b and C3c. Both C3 and C1q have been reported to be transcribed and translated at higher levels in DN [19]. Furthermore, in DM patients, those with DN had larger proportions of C1q kidney deposition than those without DN [20]. Also, C3a receptor antagonists ameliorates endothelial–myofibroblast transition (EndMT) and inflammation in DN [13,21]. For these reasons, the present study has investigated the deposition of C1q and C3c in renal histological specimens from DN patients, as well as its association with clinical and pathological parameters.

Materials and methods

Patients

A total of 277 consecutive patients with renal biopsy-proven DN, diagnosed between 2004 and 2017 at the Renal Division of Peking University First Hospital, were enrolled in the present study. All patients met criteria for a DM diagnosis as proposed by the American Diabetes Association (ADA) in 2017 [22], and a DN diagnosis as proposed by the Renal Pathology Society in 2010 [23]. DN was verified by its characteristic changes, including glomerular hypertrophy, thickened capillary basement membranes and nodular mesangial sclerosis on renal histology. Of the 277 patients, 110 had coexisting non-diabetes-related renal disease, such as mesangial proliferative glomerulonephritis, endocapillary proliferative glomerulonephritis, lupus nephritis and drug-induced kidney disease, and were therefore excluded. In addition, as pathological data for two other patients and clinical data for four patients were incomplete, these six were excluded as well. Ultimately, 161 patients were eligible for analysis in the present study (Fig. 1).

Clinical information

Patients' clinical data were extracted from the electronic medical records of our hospital, including age, gender, duration of DM, duration of hypertension, diabetic retinopathy (DR), diabetic neuropathy, HbA_{1c}, circulating haemoglobin, 24-h urinary protein, fasting blood glucose (FBG), triglyceride (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), serum creatinine, serum C3, serum C4 and estimated glomerular filtration rate (eGFR). The lattermost was calculated according to the Modification of Diet in Renal Disease (MDRD) study equation, modified for Chinese patients with CKD [24]. DR was diagnosed by trained ophthalmologists according to the international clinical guidelines proposed by the Global Diabetic Retinopathy Project Group [25], and peripheral diabetic neuropathy was verified using the validated Toronto Clinical Scoring System (Scores $>$ 5) [26].

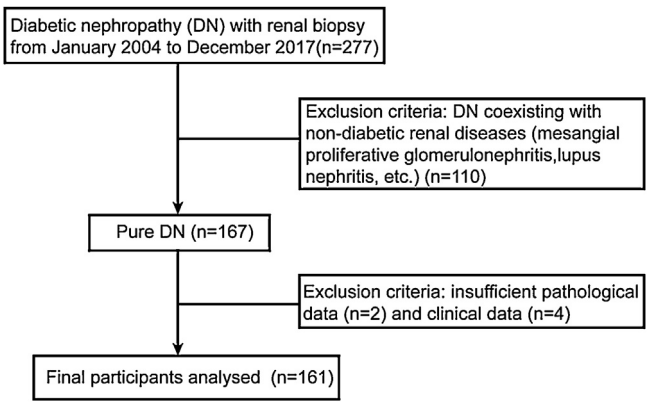

Fig. 1. Flow diagram of patient recruitment for the study.

Renal histopathology

Renal tissue specimens were assessed by direct immunofluorescence and light and electron microscopy. For the latter two investigations, a standard classification system [23] based on histological scores for glomerular lesions, tubulointerstitial lesions, vascular lesions and non-diabetic glomerular lesions was used. The presence of glomerular lesions was classified according to glomerular basement membrane thickening, mesangial expansion, nodular sclerosis (Kimmelstiel–Wilson lesions) and advanced diabetic glomerulosclerosis as found on biopsy. Interstitial fibrosis and tubular atrophy (IFTA) were scored semi quantitatively, based on the proportion of the tubulointerstitial compartment affected (0: none, 1: $<$ 25%, 2: 25–50%, 3: $>$ 50%). Interstitial inflammation (0: absent, 1: infiltration only in areas related to IFTA, 2: infiltration in areas without IFTA). Vascular lesions were scored according to the presence of arteriolar hyalinosis and large-vessel arteriosclerosis [23].

For direct immunofluorescence, the intensity of staining of the complement components (including C1q and C3c) in each renal tissue section was semi quantitatively graded on a Scale of 0–4 + (–: no fluorescence at either high or low magnification; ± : no fluorescence at low magnification, but seemingly visible at high magnification; +: seemingly visible at low magnification, but clearly visible at high magnification; ++: clearly visible at both low and high magnification; +++: clearly visible at low magnification lens, but dazzling at high magnification; and ++++: dazzling at low magnification, and even more dazzling at high magnification) [27]. All patients with $\geq$ 1+were regarded as having complement deposition. However, to compare clinical and pathological data of patients with different degrees of complement deposition, and because patients with 4+deposition of C1q or C3c are rather rare, all patients with $\geq$ 3+ deposition were classified as one group. Any scoring differences by two pathologists were repeatedly reviewed until a consensus was obtained.

This study was in compliance with the Declaration of Helsinki and approved by the ethics committees of Peking University First Hospital. Informed consent was obtained from every participant at the time of renal biopsy.

Statistical analysis

Continuous variables are presented as either the mean ± standard deviation (SD) or median and interquartile range (IQR). Categorical variables are presented as counts (n) and percentages (%). Intergroup differences between quantitative parameters were assessed by one-way analysis of variance (ANOVA) for normally distributed data. Differences in semi quantitative and quantitative parameters not normally distributed were assessed by Kruskal–Wallis or Mann–Whitney U-tests as appropriate, while differences in qualitative parameters were compared using χ^2 or Fisher's exact tests. Differences were considered significant if the P-value was $<$ 0.05. All analyses were performed by SAS version 9.4 statistical software (SAS Institute Inc., Cary, NC, USA).

Results

General data

Of our 161 patients with DN, 124 were male and 37 were female, and aged 48.6 ± 11.9 years at the time of renal biopsy. Their duration of diabetes and hypertension were 9.9 ± 6.0 years and

7.7 ± 8.5 years, respectively. In addition, 128 (79.5%), 39 (24.2%) and 44 (27.3%) of these patients also had DR, diabetic peripheral neuropathy and diabetic peripheral vascular disease, respectively. The number of glomeruli in 161 biopsy sections was 35.8 ± 14.5, with a minimum of 13 glomeruli in this cohort. A summary of clinical data from these patients is presented in Table 1.

Comparison of clinical manifestations

On direct immunofluorescence microscopy, C1q and C3c were detected in specimens from 44/161 (27.3%) and 89/161 (55.3%) patients, respectively. Of the 44 patients with C1q deposition, 38 (86.4%) also showed C3c deposition, and 27/44 (61.4%), 13/44 (29.5%) and 4/44 (9.1%) were graded as 1+, 2+ and 3+–4+, respectively, for C1q staining on a Scale of 0–4 + . C1q deposition was found in glomerular capillary walls, mesangium and tubular basement membrane (TBM) in 35/44, 37/44 and 2/44 patients, respectively. As for C3c deposition, 25/89 (28.1%), 42/89 (47.2%) and 22/89 (24.7%) were graded as 1+, 2+ and 3+–4+ on a Scale of 0–4+, respectively, with C3c deposition found in glomerular capillary walls, mesangium, Bowman's capsule and TBM in 55/89, 71/89, 3/89 and 7/89 patients, respectively. There was no significant association between location of C1q or C3c deposits and clinicopathological parameters in these DN patients.

A significantly greater proportion of men had C1q deposition vs. those without such deposits (88.84% vs. 72.65%; $P < 0.05$). In addition, patients with vs. without C1q deposition on renal histopathology had significantly lower levels of serum albumin (20.20 ± 5.50 g/L vs. 32.42 ± 6.10 g/L; $P < 0.05$) and eGFR (34.16 ± 25.21 mL/min/1.73 m^2 vs. 51.17 ± 31.56 mL/min/1.73 m^2, respectively; $P < 0.01$), but significantly higher levels of urinary protein (7.25 ± 4.20 g/24 h vs. 4.97 ± 3.76 g/24 h, respectively; $P < 0.01$) and serum creatinine (median: 209.70 μmol/L, IQR: 151.35–377.33 vs. 137.00 μmol/L, IQR: 97.70–234.20), respectively; $P < 0.01$; Table 1). Moreover, levels of circulating haemoglobin were significantly lower in patients with than without C1q deposition (104.74 ± 19.04 g/L vs. 113.01 ± 23.94 g/L, respectively; $P < 0.05$; Table 1).

Overall, there were significant differences in serum creatinine, urinary protein and eGFR ($P < 0.01$, $P < 0.01$ and $P < 0.01$, respectively) in all four groups of patients with different degrees of C1q deposition. Further analyses revealed that, compared with patients without C1q deposition, those with + C1q-stained deposition had significantly higher levels of serum creatinine and urinary protein, and lower eGFRs (all $P < 0.05$, post-hoc), while those with ++ C1q-stained deposition had significantly higher levels of urinary protein ($P < 0.05$, post-hoc; Table 2).

Patients with C3c deposition included a significantly greater proportion of men than those without C3c deposition (84.27% vs. 68.06%, respectively; $P < 0.05$). Compared with the 72 patients without C3c deposition on renal histopathology, those with such deposition had significantly higher levels of serum creatinine (median: 196.40 μmol/L, IQR: 123.50–309.85 vs. median: 128.40 μmol/L, IQR: 96.80–201.80, respectively; $P < 0.01$) and

Table 1
Baseline data in patients with and without C1q or C3c deposition on renal histology.

Variables	With C1q deposition (n = 44)	Without C1q deposition (n = 117)	With C3c deposition (n = 89)	Without C3c deposition (n = 72)
Clinical characteristics				
Age (years)	51.02 ± 10.52	47.62 ± 12.29	48.30 ± 11.77	49.28 ± 11.95
Gender (male)	39 (88.84%)[a]	85 (72.65%)	75 (84.27%)[a]	49 (68.06%)
T2D/T1D	42/2	110/7	82/7	70/2
Duration of DM (years)	8.92 ± 6.75	10.28 ± 5.68	9.71 ± 6.07	10.14 ± 5.96
Duration of hypertension (years)	8.16 ± 9.35	7.64 ± 8.33	8.00 ± 8.90	7.49 ± 8.23
Diabetic retinopathy	93 (79.49%)	35 (79.55%)	72 (80.90%)	56 (77.78%)
Diabetic neuropathy	27 (23.48%)	12 (27.91%)	22 (25.29%)	17 (23.94%)
Fasting blood glucose (mmol/L)	6.38 (5.30,8.45)	6.59 (5.48–9.18)	6.56 (5.55–8.61)	6.57 (5.18, 9.71)
Haemoglobin (g/L)	104.74 ± 19.04[a]	113.01 ± 23.94	109.56 ± 23.00	112.31 ± 23.00
D-dimer (mg/L)	0.21 (0.13,0.45)	0.18 (0.11–0.33)	0.20 (0.13–0.36)	0.17 (0.11–0.32)
Serum albumin (g/L)	20.20 ± 5.50[a]	32.42 ± 6.10	31.39 ± 5.82	32.34 ± 6.23
Serum creatinine (μmol/L)	209.70 (151.35–377.33)[b]	137.00 (97.70–234.20)	196.40 (123.50–309.85)[c]	128.40 (96.80–201.80)
Uric acid (μmol/L)	291.66 ± 92.46	392.89 ± 110.89	405.28 ± 96.66	376.82 ± 115.03
BUN (mmol/L)	23.08 (17.10–29.93)[a]	19.20 (14.40–26.00)	21.86 (16.82–29.72)[c]	17.12 (14.12–23.06)
Triglyceride (mmol/L)	1.55 (1.21–2.47)	1.71 (1.22–2.69)	1.58 (1.16–2.54)	1.72 (1.25–2.59)
Total cholesterol (mmol/L)	5.32 ± 2.10	5.18 ± 1.89	4.70 (3.84–5.97)	5.27 (4.24–6.20)
HDL cholesterol (mmol/L)	1.00 ± 0.25	1.04 ± 0.29	1.00 ± 0.23	1.06 ± 0.33
LDL cholesterol (mmol/L)	3.05 ± 1.60	2.96 ± 1.09	2.75 (2.03–3.41)	2.99 (2.09–3.74)
Urinary protein (g/24 h)	7.25 ± 4.20[b]	4.97 ± 3.76	6.04 ± 4.01	5.04 ± 3.94
HbA_{1c} (%)	6.20 (5.70–6.90)[a]	6.90 (5.90–8.10)	6.50 (5.80–7.60)	7.00 (5.90–8.00)
Serum C3 (g/L)	0.90 (0.77–1.09)	0.89 (0.81–1.05)	0.89 (0.78–1.02)	0.92 (0.83–1.07)
Serum C4 (g/L)	0.27 (0.21–0.31)	0.25 (0.20–0.30)	0.25 (0.20–0.29)	0.26 (0.20–0.32)
ESR (mm/1 h)	55.75 ± 30.68	51.80 ± 33.87	55.02 ± 32.77	50.34 ± 33.21
eGFR (mL/min/1.73 m^2)[d]	34.16 ± 25.21[b]	51.17 ± 31.56	40.09 ± 27.97[c]	54.48 ± 32.49
CKD stage (1/2/3a/3b/4/5)	2/2/7/9/13/11[b]	15/28/20/17/24/13	6/14/12/14/28/15[c]	11/16/15/12/9/9
Pathological characteristics				
Glomerular class (I/IIa/IIb/III/IV)	0/5/28/9/2	1/15/74/8/19	0/13/51/14/11	1/7/51/3/10
IFTA Score (0/1/2/3)	0/4/31/9[b]	6/35/69/7	2/16/59/12[c]	4/23/41/4
Interstitial inflammation Score (0/1/2)	0/11/33[#]	8/41/68	1/28/60	7/24/41
Vascular lesion Score (0/1/2)	6/20/18[a]	40/43/34	21/37/31	25/26/21
Global sclerosis (%)	29.0% ± 22.0%	24.0% ± 20.0%	30.0% ± 21.0%[c]	20.0% ± 19.0%

Continuous variables are presented as means ± standard deviation or medians (interquartile range), categorical variables as n (%).
T1D/T2D: type 1/type 2 diabetes; DM: diabetes mellitus; BUN: blood urea nitrogen; HDL/LDL: high-density/low-density lipoprotein; ESR: erythrocyte sedimentation rate; eGFR: estimated glomerular filtration rate; CKD: chronic kidney disease; IFTA: interstitial fibrosis and tubular atrophy.
[a] Two-tailed $P < 0.05$.
[b] Two-tailed $P < 0.01$ (with vs. without C1q deposition).
[c] Two-tailed $P < 0.01$ (with vs. without C3c deposition).
[d] 175 × plasma creatinine$^{-1.234}$ × age$^{-0.179}$ × 0.79 (if female).

Table 2
Clinical and pathological characteristics of patients with different degrees of C1q or C3c deposition in kidney tissue.

Variables	C1q − to ± (*n* = 117)	C1q + (*n* = 27)	C1q ++ (*n* = 13)	C1q +++ to ++++ (*n* = 4)
Clinical characteristics				
Serum creatinine (μmol/L)[a]	137.00 (97.70–234.20)	212.90 (140.20–491.70)	179.30 (145.00–267.00)	255.88 (166.40–395.50)
Urinary protein (g/24 h)[a]	4.98 ± 3.76	7.25 ± 3.46	7.67 ± 5.85	5.89 ± 2.12
eGFR (mL/min/1.73 m^2)[a,d]	51.18 ± 31.56	34.63 ± 28.89	35.57 ± 19.76	26.42 ± 14.94
Pathological characteristics				
Glomerular class (I/IIa/IIb/III/IV)	1/15/74/8/19	0/3/16/6/2	0/2/10/1/0	0/0/2/2/0
IFTA Score (0/1/2/3)[a]	6/35/69/7	0/3/17/7	0/1/10/2	0/0/4/0
Interstitial inflammation Score (0/1/2)	8/41/68	0/9/18	0/2/11	0/0/4
Vascular lesion Score (0/1/2)	40/43/34	5/12/10	1/5/7	0/3/1
Global sclerosis (%)	24.0 ± 20.0	30.0 ± 24.0	24.0 ± 18.0	42.0 ± 19.0
Variables	C3c − to ± (*n* = 72)	C3c + (*n* = 25)	C3c ++ (*n* = 42)	C3c +++ to ++++ (*n* = 22)
Clinical characteristics				
Serum creatinine (μmol/L)[b]	128.40 (96.80–201.80)	199.00 (114.70–264.10)	193.05 (131.20–320.90)	186.40 (121.50–285.90)
Urinary protein (g/24 h)	5.04 ± 3.94	5.50 ± 4.35	6.33 ± 4.16	6.03 ± 3.51
eGFR (mL/min/1.73 m^2)[b,d]	54.48 ± 32.50	42.15 ± 29.30	39.05 ± 28.28	39.74 ± 27.02
Pathological characteristics				
Glomerular class (I/IIa/IIb/III/IV)	1/7/51/3/10	0/3/16/2/4	0/8/21/7/6	0/2/14/5/1
IFTA Score (0/1/2/3)[b]	4/23/41/4	1/3/19/2	0/7/27/8	1/6/13/2
Interstitial inflammation Score (0/1/2)	7/24/41	1/7/17	0/14/28	0/7/15
Vascular lesion Score (0/1/2)	25/26/21	6/7/12	10/19/13	5/11/6
Global sclerosis (%)[c]	20.0 ± 19.0	27.0 ± 23.0	33.0 ± 19.0	28.0 ± 21.0

Continuous variables are presented as means ± standard deviation or medians (interquartile range), categorical variables as *n* (%); one patient was C3c ++++ ; no patients were C1q ++++ .
eGFR: estimated glomerular filtration rate; IFTA: interstitial fibrosis and tubular atrophy.
[a] Two-tailed $P < 0.01$ (between different degrees of C1q deposition).
[b] Two-tailed $P < 0.05$.
[c] Two-tailed $P < 0.01$ (between different degrees of C3c deposition).
[d] 175 × plasma creatinine$^{-1.234}$ × age$^{-0.179}$ × 0.79 (if female).

lower eGFR (40.09 ± 27.97 mL/min/1.73 m^2 vs. 54.48 ± 32.49 mL/min/1.73 m^2, respectively; $P < 0.01$) at the time of renal biopsy. There was no significant difference in urinary protein levels between patients with and without C3c deposition (Table 1).

However, there were significant differences in serum creatinine and eGFR (both $P < 0.05$) across all four groups of patients with different degrees of C3c deposition. Further analysis also revealed that, compared with patients without C3c deposition, those with ++ C3c-stained deposition had significantly higher serum creatinine levels ($P < 0.05$, post-hoc) and lower eGFRs ($P < 0.05$, post-hoc; Table 2).

On further investigations of patients with both C1q and C3c deposition vs. those with only one or no complement deposition, significant differences were found for serum creatinine, urinary protein and eGFR ($P < 0.01$, $P < 0.01$ and $P < 0.01$, respectively) in the four study groups (Table 3). In particular, on comparing patients with only C3c deposition vs. those with deposits of both complement proteins, the latter showed significantly higher levels of both serum creatinine and urinary protein, but lower eGFRs (all $P < 0.05$, post-hoc).

Comparison on renal histopathology

Comparing patients without vs. with C1q deposition, the latter had significantly higher IFTA, interstitial inflammation and vascular lesion Scores ($P < 0.01$, $P < 0.05$ and $P < 0.05$, respectively). Patients with C1q deposition also had a significantly greater proportion of IgM deposition (95.45% vs. 56.41%, respectively; $P < 0.01$; Table 1).

Overall, there was a significant difference in IFTA Score ($P < 0.01$) across all four groups of patients with different degrees of C1q deposition. Further analyses also revealed that, compared with no C1q deposition, patients who had + C1q-stained deposition had significantly higher IFTA Scores ($P < 0.05$, post-hoc; Table 2).

On the other hand, there were no significant differences in glomerular class or interstitial inflammation and vascular lesion Scores between patients with and without C3c deposition. Comparing patients without vs. with C3c deposition on renal histopathology, the latter had significantly higher IFTA Scores ($P < 0.01$), and significantly greater proportions of global sclerosis (30.0% ± 21.0% vs. 20.0% ± 19.0%, respectively; $P < 0.01$) and IgM deposition (82.08% vs. 48.61%, respectively; $P < 0.01$; Table 2). There were also significant differences in IFTA Scores and percentages of global sclerosis ($P < 0.05$ and $P < 0.01$, respectively) across all four groups with different degrees of C3c deposition, while further analyses found that, compared with patients with no C3c deposits, those with ++ C3c-stained deposition had significantly higher IFTA Scores and greater proportions of global sclerosis (all $P < 0.05$, post-hoc; Table 2).

On investigating patients with both C1q and C3c deposition in comparison to those with only one or no complement deposition, significant differences in IFTA Scores and percentages of global sclerosis ($P < 0.01$ and $P < 0.05$, respectively) were found in all four groups (Table 3). More specifically, compared with patients with only C3c deposition, those with both complement proteins had significantly higher IFTA Scores ($P < 0.05$, post-hoc).

Discussion

The development of DN is generally thought to be associated with hyperglycaemia, disturbances of carbohydrates and lipids, and hypertension, all of which lead to glomerular lesions. However, a growing number of studies indicate that inflammation and immunity are also involved in the pathogenesis of DN [8–10]. As the complement system is an important part of immune system, regulating both innate and acquired immunity, emerging evidence now suggests that it is involved in the development of DN: endothelial dysfunction can be caused by complement activation, which increases the permeability of

Table 3
Characteristics of patients according to C3c and/or C1q deposition.

Variables	Both C3c and C1q deposition	Only C1q deposition	Only C3c deposition	No C3c and C1q deposition
n (%)	38 (23.6)	6 (3.7)	51 (31.7)	66 (41.0)
Clinical characteristics				
Serum creatinine (μmol/L)[b]	219.95 (161.30–417.00)	151.35 (129.20–214.50)	181.50 (99.00–264.10)	126.92 (9660–194.20)
Urinary protein (g/24 h)[b]	7.04 ± 4.15	8.85 ± 4.70	5.29 ± 3.79	4.74 ± 3.75
eGFR (mL/min/1.73 m^2)[b,c]	32.09 ± 24.21	47.31 ± 29.76	46.06 ± 29.31	55.14 ± 32.87
Pathological characteristics				
Glomerular class (I/IIa/IIb/III/IV)	0/5/23/9/1	0/0/5/0/1	0/8/28/5/10	1/7/46/3/9
IFTA Score (0/1/2/3)[b]	0/3/27/8	0/1/4/1	2/13/32/4	4/22/37/3
Interstitial inflammation Score (0/1/2)	0/9/29	0/2/4	1/19/31	7/22/37
Vascular lesion Score (0/1/2)	5/19/14	1/1/4	16/18/17	24/25/17
Global sclerosis (%)[a]	30.0% ± 21.0%	26.0% ± 29.0%	30.0% ± 21.0%	20% ± 18%

Continuous variables are presented as means ± standard deviation or medians (interquartile range), categorical variables as *n* (%).
eGFR: estimated glomerular filtration rate; IFTA: interstitial fibrosis and tubular atrophy.
[a] Two-tailed $P < 0.05$.
[b] Two-tailed $P < 0.01$ (all four groups).
[c] $175 \times \text{plasma creatinine}^{-1.234} \times \text{age}^{-0.179} \times 0.79$ (if female).

glomeruli [28], while the complement cascade can promote inflammation-mediated vascular damage [29]; also, in animal models of DN, complement inhibition mitigated albuminuria and glomerular damage [13,15,21,30].

In the present study, clinical and histological parameters in DN patients with and without C1q or C3c kidney deposition were compared, and found that patients with C1q deposition in kidney tissue had more severe renal lesions than those without C1q deposition, along with significantly lower levels of serum albumin and eGFR, but higher levels of urinary protein and serum creatinine, and higher scores for IFTA and vascular lesions. These results highlight the significance of the classic DN pathway, for which certain clues had already suggested a potential role in DN pathogenesis [19,20,31–35]: for example, C1q messenger RNA (mRNA) expression is upregulated in the glomeruli and tubulointerstitium in DN patients [19], while C1q protein deposits were increased in certain kidney structures (such as glomeruli hili and arterioles) and associated with the presence of DN [20]. Also, as the antigen–antibody immune complex can activate the classic complement pathway, the fact that patients with C1q deposits also had significantly greater proportions of IgM deposition was not surprising. In fact, Bus et al. [20] found that glomerular IgM deposits were co-localized with C1q, while C1q was reported to bind with advanced glycation end products (AGEs) [34] and modified LDL [33], which could also release growth factors and cytokines through the classic pathway in DN[36,37]. Moreover, higher levels of oxidized (OX) LDL and AGE LDL in the immune complex have been associated with progression of albuminuria and DN [32,38].

One finding of the present study was that patients with C3c kidney deposition had more severe renal lesions than those without C3c deposits, along with significantly higher levels of serum creatinine, higher IFTA Scores and larger proportions of global sclerosis, but lower eGFRs. In fact, kidney deposition of C3 suggests complement activation of the common pathway, making it of interest to further investigate complement pathways other than the classic one activated in DN.

More than half of our patients with C1q deposition had C3c deposition, indicating that the classic pathway was activated in DN. However, because of the non-covalent combination of C1q with renal cells, activation of this pathway is difficult to detect. Another reason may be that pathways other than the classic one are activated, and the different pathways may have various degrees of involvement in the development of DN. Indeed, previous studies have suggested that activation of the alternative and MBL pathways may also be participating in the pathogenesis of DN [31,39,40]. However, the exact role of the classic pathway in DN still needs further investigation.

Although no statistically significant difference was found in serum C3 levels in patients with or without C3c deposition in the kidney, our study did detect patients with C3 deposition who had visibly lower levels of circulating C3 (median: 0.89 g/L, IQR: 0.78–1.02 vs. 0.92 g/L, IQR: 0.83–1.07) than those without C3 deposits. These reduced circulating levels of C3 in DN might be explained by complement activation resulting in excessive consumption of C3 and kidney deposition or urinary protein excretion (proteinuria). However, a study by Rasmussen et al. [17] found that high concentrations of C3 were associated with an increased risk of DN, which is not consistent with our present findings.

Our present study has some limitations. First, as an observational study, it cannot identify any causal relationship between complement deposition and DN and, second, whether complement is activated locally in the kidneys or systemically in DN has yet to be completely elucidated.

In conclusion, deposition of C1q and C3c on renal histopathology evaluation was associated with more severe kidney damage in DM patients with DN. Nevertheless, further investigations are still needed to determine the precise role of complement in the development of DN.

Disclosures

All of the authors declare no competing interests.

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References

[1] Zhang JJ, Yang L, Huang JW, Liu YJ, Wang JW, Zhang LX, et al. Characteristics and comparison between diabetes mellitus and non-diabetes mellitus among chronic kidney disease patients: a cross-sectional study of the chinese cohort study of chronic kIDNEY disease (C-STRIDE). Oncotarget 2017;8:106324–32.
[2] Zhang L, Long J, Jiang W, Shi Y, He X, Zhou Z, et al. Trends in chronic kidney disease in China. N Engl J Med 2016;375:905–6.
[3] Quinn M, Angelico MC, Warram JH, Krolewski AS. Familial factors determine the development of diabetic nephropathy in patients with IDDM. Diabetologia 1996;39:940–5.
[4] Krolewski AS, Warram JH, Christlieb AR, Busick EJ, Kahn CR. The changing natural history of nephropathy in type I diabetes. Am J Med 1985;78:785–94.

[5] The Microalbuminuria Collaborative Study Group. Predictors of the development of microalbuminuria in patients with Type 1 diabetes mellitus: a seven-year prospective study. Diabet Med 1999;16:918–25.
[6] Gall MA, Hougaard P, Borch-Johnsen K, Parving HH. Risk factors for development of incipient and overt diabetic nephropathy in patients with non-insulin dependent diabetes mellitus: prospective, observational study. Bmj 1997;314:783–8.
[7] Ravid M, Brosh D, Ravid-Safran D, Levy Z, Rachmani R. Main risk factors for nephropathy in type 2 diabetes mellitus are plasma cholesterol levels, mean blood pressure, and hyperglycemia. Arch Intern Med 1998;158:998–1004.
[8] Yang L, Brozovic S, Xu J, Long Y, Kralik PM, Waigel S, et al. Inflammatory gene expression in OVE26 diabetic kidney during the development of nephropathy. Nephron Exp Nephrol 2011;119:e8–20.
[9] Navarro-Gonzalez JF, Mora-Fernandez C. The role of inflammatory cytokines in diabetic nephropathy. J Am Soc Nephrol 2008;19:433–42.
[10] Saraheimo M, Teppo AM, Forsblom C, Fagerudd J, Groop PH. Diabetic nephropathy is associated with low-grade inflammation in Type 1 diabetic patients. Diabetologia 2003;46:1402–7.
[11] Schreiber RD, Morrison DC, Podack ER, Müller-Eberhard HJ. Bactericidal activity of the alternative complement pathway generated from 11 isolated plasma proteins. J Exp Med 1979;149:870–82.
[12] Watanabe S, Tomino Y, Inoue W, Yagame M, Kaneshige H, Nomoto Y, et al. Detection of immunoglobulins and/or complement in kidney tissues from non-obese diabetic (NOD) mice. Tokai J Exp Clin Med 1987;12:201–8.
[13] Li L, Yin Q, Tang X, Bai L, Zhang J, Gou S, et al. C3a receptor antagonist ameliorates inflammatory and fibrotic signals in type 2 diabetic nephropathy by suppressing the activation of TGF-beta/smad3 and IKBalpha pathway. PLoS One 2014;9:e113639.
[14] Kelly KJ, Liu Y, Zhang J, Dominguez JH. Renal C3 complement component: feed forward to diabetic kidney disease. Am J Nephrol 2015;41:48–56.
[15] Fujita T, Ohi H, Komatsu K, Endo M, Ohsawa I, Kanmatsuse K. Complement activation accelerates glomerular injury in diabetic rats. Nephron 1999;81:208–14.
[16] Zhang J, Wang Y, Zhang R, Li H, Han Q, Guo R, et al. Implication of decreased serum complement 3 in patients with diabetic nephropathy. Acta Diabetol 2018;55:31–9.
[17] Rasmussen KL, Nordestgaard BG, Nielsen SF. Complement C3 and risk of diabetic microvascular disease: a cohort study of 95,202 individuals from the general population. Clin Chem 2018;64:1113–24.
[18] Ehrnthaller C, Ignatius A, Gebhard F, Huber-Lang M. New insights of an old defense system: structure, function, and clinical relevance of the complement system. Mol Med 2011;17:317–29.
[19] Woroniecka KI, Park AS, Mohtat D, Thomas DB, Pullman JM, Susztak K. Transcriptome analysis of human diabetic kidney disease. Diabetes 2011;60:2354–69.
[20] Bus P, Chua JS, Klessens CQF, Zandbergen M, Wolterbeek R, van Kooten C, et al. Complement activation in patients with diabetic nephropathy. Kidney Int Rep 2018;3:302–13.
[21] Li L, Chen L, Zang J, Tang X, Liu Y, Zhang J, et al. C3a and C5a receptor antagonists ameliorate endothelial–myofibroblast transition via the Wnt/beta-catenin signaling pathway in diabetic kidney disease. Metabolism 2015;64:597–610.
[22] American Diabetes Association. Standards of medical care in diabetes-2017 abridged for primary care providers. Clin Diabetes 2017;35:5–26.
[23] Tervaert TW, Mooyaart AL, Amann K, Cohen AH, Cook HT, Drachenberg CB, et al. Pathologic classification of diabetic nephropathy. J Am Soc Nephrol 2010;21:556–63.
[24] Ma YC, Zuo L, Chen JH, Luo Q, Yu XQ, Li Y, et al. Modified glomerular filtration rate estimating equation for Chinese patients with chronic kidney disease. J Am Soc Nephrol 2006;17:2937–44.
[25] Wilkinson CP, Ferris 3rd , Klein RE, Lee PP, Agardh CD, Davis M, et al. Proposed international clinical diabetic retinopathy and diabetic macular edema disease severity scales. Ophthalmology 2003;110:1677–82.
[26] Perkins BA, Olaleye D, Zinman B, Bril V. Simple screening tests for peripheral neuropathy in the diabetes clinic. Diabetes Care 2001;24:250–6.
[27] Jennette JC, D'Agati VD, Olson JL. Heptinstall's pathology of the kidney. Philadelphia: Lippincott Williams & Wilkins; 2006.
[28] Tedesco F, Fischetti F, Pausa M, Dobrina A, Sim RB, Daha M. Complement-endothelial cell interactions: pathophysiological implications. Mol Immunol 1999;36:261–8.
[29] Markiewski MM, Lambris JD. The role of complement in inflammatory diseases from behind the scenes into the spotlight. Am J Pathol 2007;171:715–727.
[30] Wang H, Vinnikov I, Shahzad K, Bock F, Ranjan S, Wolter J, et al. The lectin-like domain of thrombomodulin ameliorates diabetic glomerulopathy via complement inhibition. Thromb Haemost 2012;108:1141–53.
[31] Li XQ, Chang DY, Chen M, Zhao MH. Complement activation in patients with diabetic nephropathy. Diabetes Metab 2018. http://dx.doi.org/10.1016/j.diabet.2018.04.001 [pii: S1262-3636(18)30078-8; Epub ahead of print].
[32] Atchley DH, Lopes-Virella MF, Zheng D, Kenny D, Virella G. Oxidized LDL-anti-oxidized LDL immune complexes and diabetic nephropathy. Diabetologia 2002;45:1562–71.
[33] Biro A, Thielens NM, Cervenák L, Prohászka Z, Füst G, Arlaud GJ. Modified low-density lipoproteins differentially bind and activate the C1 complex of complement. Mol Immunol 2007;44:1169–77.
[34] Chikazawa M, Shibata T, Hatasa Y, Hirose S, Otaki N, Nakashima F, et al. Identification of C1q as a binding protein for advanced glycation end products. Biochemistry 2016;55:435–46.
[35] Uesugi N, Sakata N, Nangaku M, Abe M, Horiuchi S, Hisano S, et al. Possible mechanism for medial smooth muscle cell injury in diabetic nephropathy: glycoxidation-mediated local complement activation. Am J Kidney Dis 2004;44:224–38.
[36] Forbes JM, Cooper ME, Oldfield MD, Thomas MC. Role of advanced glycation end products in diabetic nephropathy. J Am Soc Nephrol 2003;14:254–8.
[37] Saad AF, Virella G, Chassereau C, Boackle RJ, Lopes-Virella MF. OxLDL immune complexes activate complement and induce cytokine production by MonoMac 6 cells and human macrophages. J Lipid Res 2006;47:1975–83.
[38] Lopes-Virella MF, Carter RE, Baker NL, Lachin J, Virella G, DCCT/EDIC Research Group. High levels of oxidized LDL in circulating immune complexes are associated with increased odds of developing abnormal albuminuria in Type 1 diabetes. Nephrol Dial Transplant 2012;27:1416–23.
[39] Ostergaard J, Thiel S, Gadjeva M, Hansen TK, Rasch R, Flyvbjerg A. Mannose-binding lectin deficiency attenuates renal changes in a streptozotocin-induced model of type 1 diabetes in mice. Diabetologia 2007;50:1541–9.
[40] Hovind P, Hansen TK, Tarnow L, Thiel S, Steffensen R, Flyvbjerg A, et al. Mannose-binding lectin as a predictor of microalbuminuria in type 1 diabetes: an inception cohort study. Diabetes 2005;54:1523–7.