Clinicopathological Spectrum of Cryoglobulinemic Glomerulonephritis without Evidence of Autoimmunity Disorders: A Retrospective Study from a Single Institute of China

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Cryoglobulinemic glomerulonephritis · Clinicopathological spectrum · Monoclonal gammopathy · Renal prognosis

Abstract
Background: Cryoglobulinemic glomerulonephritis (Cryo-GN), caused by circulating cryoglobulins, has varied etiology and clinical-pathologic manifestations. This study aimed to investigate the clinicopathological spectrum and outcome of patients with various Cryo-GN in China. Methods: A retrospective review of 74 Chinese patients with biopsy-proven cryoglobulin-related renal lesions in Peking University First Hospital from 2010 to 2020 was performed. Results: The mean age at diagnosis was 52.9 ± 15.0 years, and the female-to-male ratio was about 2/5. For the etiology screening, serum/urine monoclonal immunoglobulin could be detected on immunofixation electrophoresis in 34% of patients, including 6 patients who had hematological malignancies. Fifty-seven percent of patients had HBV infection, far more than HCV infection (5%). Ten percent of patients had other infections, and 27% of patients were classified as essential or idiopathic. Eleven out of the 15 patients with type II cryoglobulinemia had a consistent monotype of serum monoclonal immunoglobulins and monoclonal cryoprecipitate. The clinical manifestations were similar between various types of cryoglobulinemia. Hematuria, proteinuria, hypertension, anemia, and chronic renal insufficiency were the most common features. Fifty-three percent of patients presented with nephrotic syndrome, and 32% experienced acute kidney injury. Hypocomplementemia, serum-positive rheumatoid factor activity, and skin lesions were reported in 45%, 29%, and 28% of patients, respectively. After a median of 24 months follow-up, 18 patients reached end-stage kidney disease. The clone-targeted treatment could retard the renal deterioration compared with immunosuppressive therapy. Conclusions: This was the largest single-center, clinicopathological retrospective study from a single institute in China.
study of Cryo-GN in China. Our data strongly support the association between monoclonal gammopathy and type II Cryo-GN. The renal responsive rate of immunosuppressant therapy is still suboptimal. The clone-targeted treatment shows promising effects in patients with type I or II Cryo-GN.

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Introduction

Cryoglobulins are immunoglobulins (Ig) that precipitate at temperatures less than 37°C and dissolve after rewarming [1], which could be grouped into three types based on the clonality and type of immunoglobulins [2]. Type I cryoglobulins are composed of a single monoclonal Ig. Type II and type III cryoglobulins, also referred to as mixed cryoglobulinemia, are immune complexes with rheumatoid factor (RF) activity. Type II cryoglobulins contain a monoclonal component, mostly of IgM isotype and polyclonal IgG; while type III cryoglobulins are polyclonal IgM and polyclonal IgG [3–7]. Cryoglobulinemia, defined by the presence of circulating cryoglobulins, is a clinical condition that may be asymptomatic or presents with systemic inflammatory syndromes [8, 9]. The kidney is one of the most easily involved organs targeted by pathogenic cryoglobulins [6, 7]. Cryoglobulinemic glomerulonephritis (Cryo-GN) is the principal manifestation of renal involvement, usually manifesting as varying degrees of microhematuria, proteinuria (UTP), hypertension, and/or renal failure [10]. However, the difference in clinical and renal histopathological features among different types of cryoglobulinemia has not been fully elucidated. The present study retrospectively identified the serum type of cryoglobulins and the renal histopathological spectrum of Cryo-GN from Peking University First Hospital. We aimed to investigate the pathogenesis, clinical and histopathological features, treatments, and outcomes of the patients with biopsy-proven Cryo-GN in China.

Method

Patients

All patients (n = 2,435) with positive serum cryoglobulin tests in Peking University First Hospital between 2010 and 2020 were identified for further investigation. The electronic medical records were reviewed to determine those who had a renal biopsy performed. After which, 1,152 patients with renal biopsy results were enrolled for pathological assessments. Two renal pathologists reviewed the renal biopsies independently to screen for the Cryo-GN. The inclusion criteria were one of the following: (1) ultrastructurally organized tubular, cylindrical, or crystalloid organization on electron microscopy; (2) intraluminal pseudo-thrombi/wire-loop on light microscopy; (3) renal lesion patterns of membranous proliferative glomerulonephritis (MPGN) or endocapillary proliferative glomerulonephritis without other proven pathogenesis such as acute poststreptococcal glomerulonephritis, lupus nephritis, IgA nephropathy, C3 glomerulopathy, or proliferative glomerulonephritis and monoclonal immunoglobulin deposits. Patients with confirmed autoimmune diseases, such as systemic lupus erythematosus, rheumatoid arthritis, or Sjögren’s syndrome, were excluded from further analysis because they have particular pathogenesis, treatment methods, and clinical course (Fig. 1).

Clinical and Laboratory Assessment during Follow-Up

The electronic medical records were reviewed to collect the demographic characteristics, past medical history, clinical manifestations, and laboratory tests results. Extra-renal manifestations, including skin purpura, arthralgia, and Raynaud’s phenomenon, were recorded through physical examination. Laboratory tests included leukocytes, hemoglobin, platelet count, serum creatinine, UTP, hematuria, serum albumin (Alb), complements, immunoglobulins, serum/urine immunofixation electrophoresis (IFE), HCV antibody, HCV-RNA, HBV antigens and antibodies, and HBV DNA. The detection of serum cryoglobulins was carried out as previously reported (online suppl. Methods; for all online suppl. material, see www.karger.com/doi/10.1159/000522537) [11]. Hematuria was divided into gross and microscopic hematuria. Microscopic hematuria was defined as more than three erythrocytes per high-power field (HPF) in urine sediment under microscopy and grouped according to the degree of severity: mild: 3–10 erythrocytes per HPF; moderate: 10–100 erythrocytes per HPF; and severe: more than 100 erythrocytes per HPF. The estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease (CKD) Epidemiology Collaboration equation. CKD stage and acute kidney injury (AKI) are defined as KIDGO guidelines line states. Anemia is determined using the WHO criteria. Treatment options were also reviewed, including supportive, antiviral, immunosuppressant, and clone-targeted chemotherapy. Patients were followed up in the outpatient clinic. Treatment response and renal endpoint (end-stage kidney disease) were collected. Renal responses to treatment were defined as the follows: (1) complete remission reduction of UTP to <0.3 g/24 h, stable serum creatinine and serum Alb >35 g/L; (2) partial remission: reduction of UTP to 0.3–<3.5 g/24 h and a decrease >50% from baseline; (3) no response: not reaching PR.

Renal Histopathology Assessment

Renal biopsy was examined by standard direct immunofluorescence, light microscopy, and electron microscopy. Glass slides were reviewed by two pathologists independently. Intraluminal pseudo-thrombi, wire-loop, and substructure on electron microscopy were evaluated as the divergent categorical score. The degree of global glomerulosclerosis, cellular/fibro-cellular crescents, and interstitial fibrosis and tubular atrophy were also evaluated. Differences in scoring between the two pathologists were resolved by reviewing the biopsies to reach a consensus.

Statistical Analysis

SPSS software (version 22; SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Continuous data were expressed as mean ± SD and compared using Student’s t test for normally dis-
Fig. 1. Study flowchart of patients with cryoglobulinemia who underwent a kidney biopsy from 2010 to 2020. AASV, ANCA-associated small vasculitis; AIN, acute interstitial nephritis; anti-GBM, antglomerular basement membrane; aMN, atypical membranous nephropathy; Cryo-GN, cryoglobulinemic glomerulonephritis; C3G, C3 glomerulopathy; CSH, cryo-globulinemic glomerulonephritis; TMA, thrombotic microangiopathy; TIN, tubular-interstitial nephritis; TBMN, thin basement membrane nephropathy; LN, lupus nephritis; MIDD, monoclonal immunoglobulin deposition disease; MCD, minimal change disease; MN, membranous glomerulonephritis; MsPGN, mesangial proliferative glomerulonephritis; PGNMID, membranous proliferative glomerulonephritis; HSPN, Henoch–Schönlein purpura nephritis; IgAN, IgA nephropathy; LCCN, light-chain cast nephropathy; LCPT, light-chain proximal tubulopathy; LN, lupus nephritis; MCD, minimal change disease; MN, membranous glomerulonephritis; MIDD, monoclonal immunoglobulin deposition disease; MPGN, membranous proliferative glomerulonephritis; MsPGN, mesangial proliferative glomerulonephritis; PGNMID, membranous proliferative glomerulonephritis and monoclonal immunoglobulin deposits; TBMN, thin basement membrane nephropathy; TIN, tubular-interstitial nephropathy; TMA, thrombotic microangiopathy.

Distributed variables. Non-normally distributed variables were expressed as median with range and compared using the Mann-Whitney U test. Differences between groups of semiquantitative data were tested with the Mann-Whitney U test. Categorical variables were presented as proportions and compared using the χ² test. All p values are two-sided, and statistical significance is considered as p < 0.05. Survival analyses were performed using the Kaplan-Meier analysis and Cox proportional hazards models as appropriate.

Results

Patient and Clinical Features
Etiology Assessments

After pathological assessments, 74 patients were included in the investigation and divided into four groups according to Brouet’s classification, as shown in the Table 1. Notably, the type of cryoglobulinemia in 18 patients (24%) could not be determined by IFE due to the low concentration of serum cryoglobulins. The distribution of monoclonal components of cryoprecipitate in type I cryoglobulinemia was IgMκ (n = 4, 40%), IgGκ (n = 3, 30%), and IgGλ (n = 3, 30%). The distribution of monoclonal components in type II cryoglobulinemia was as follows: IgMκ (n = 17, 70%), IgGκ (n = 3, 13%), IgGλ (n = 3, 13%), and IgAλ (n = 1, 4%) (online suppl. Table S1). Monoclonal Ig could be detected by serum/urine IFE in 34% (n = 25) of patients, mainly in the patients with type I (n = 6) or type II (n = 15) cryoglobulinemia. Eleven out of 15 patients with type II cryoglobulinemia had consistent isotypes of serum monoclonal Ig and cryoprecipitates (IgMκ = 5, IgGκ = 3, IgGκ = 2, IgAλ = 1) (online suppl. Table S1). Six patients had hematological malignancies, including chronic B lymphocyte leukemia/small B-cell lymphoma (n = 2, type I), POMES syndrome (n = 1, type II), lymphoplasmacytic lymphoma (n = 1, type I), and multiple myeloma (n = 2, type I and type III). 57.1% of patients had HBV infection, e-
ther active infection (HBsAg+, \(n = 15\)) or previous infection (HBsAg−/HBcAb+, \(n = 25\)), far more than that of HCV infection \((n = 4)\). Monoclonal spike was also detected on IFE in 9 patients with active hepatic viral infection \((HBV = 6, HCV = 3)\), including seven with type II cryoglobulinemia and monoclonal gammopathy of undetermined significance, and one with type I cryoglobulinemia and lymphoplasmacytic lymphoma. Seven patients \(10\%\) had other viral \((CMV, EBV, HSV)\) infections or bacterial infections. Twenty patients \(26\%\) were classified as having essential cryoglobulinemia after a thorough examination, which was more likely to occur in the patients with type III or undetermined type cryoglobulinemia.

Demographic and Clinical Characteristics

As shown in Table 2, the mean age of diagnosis was 53 ± 15 years, and the female-to-male ratio was about 2/5, of which the situation was similar among the four groups. Hypertension \((n = 54, 73\%)\) and anemia \((n = 60, 81\%)\) were the most common comorbidities among the patients. Diabetes was highest in the type I cryoglobulinemia group. The ex-renal manifestations occurred in 28% \((n = 21)\) of patients, including skin purpura \((n = 18)\), arthralgia \((n = 5)\), and Raynaud’s phenomenon \((n = 3)\). All patients had autoimmune antibodies screened, which turned out negative in 61 patients. Eleven patients had only positive antinuclear antibodies (ANA) with a titer of 1:100. One patient had positive PNCA antibody with ANA 1:320, and one patient had positive Sm antibody and U1RNP antibody with ANA 1:3,200. Still, neither of them met the diagnosis criteria of SLE even after long-term follow-up. Hypocomplementemia occurred in 45% \((n = 33)\) of patients, including 7% \((n = 5)\) who had isolated decreased C4 levels and 24% \((n = 18)\) who had a combined decrease in C4 and C3 levels. Positive RF activity was found in 29% \((18/62)\) patients. No significant difference was reported in the clinical parameters between the four groups.

### Table 1. The pathogenic assessments of various Cryo-GN

|                           | Total \((n = 74)\) | Type I \((n = 10)\) | Type II \((n = 24)\) | Type III \((n = 22)\) | Unknown type \((n = 18)\) | \(p\) value |
|---------------------------|-------------------|---------------------|---------------------|---------------------|---------------------|------------|
| Serum/urine monoclonal Ig, \(n(\%)\) | 25 (33.8) | 6 (60.0) | 15 (62.5) | 1 (4.5) | 3 (16.7) | <0.001 |
| \(\text{IgGa} = 7 \text{IgAλ} = 1\) | \(\text{IgGa} = 3\) | \(\text{IgGa} = 4 \text{IgAλ} = 1\) | \(\text{IgGa} = 2\) | \(\text{IgGa}, \text{λ} = 1\) | \(\text{IgGa} = 1\) | \(\lambda = 2\) |
| \(\text{IgGκ} = 6 \text{κ} = 1\) | \(\text{IgGκ} = 1\) | \(\text{IgGκ} = 1\) | \(\text{IgGκ} = 1\) | \(\text{IgGκ} = 1\) | \(\text{IgGκ} = 1\) | \(\lambda = 2\) |
| \(\lambda = 3 \text{IgGκ, λ} = 1^*\) | 6 (8.1) | 4 (40.0) | 1 (4.2) | 1 (4.5) | 0 | 0.001 |
| Hematological malignancies, \(n(\%)\) | 4 (5.4) | 1 (10.0) | 3 (13.6) | 7 (31.8) | 9 (50.0) | 0.028 |
| CLL/SBCL = 2 | POMES = 1 | Bacteria = 1 | HSV = 1 | 0.948 |
| LPL = 1 | MM = 1 | 0.800 |
| HBV infection, \(n(\%)\) | 15 (20.3) | 2 (20.0) | 5 (20.8) | 2 (8.3) | 3 (12.5) | 0.848 |
| HbAg+ | HBCAb+ with HBsAg | 25 (36.8) | 3 (30.0) | 10 (41.7) | 2 (8.3) | 3 (13.6) | 0.948 |
| HCV infection, \(n(\%)\) | 4 (5.4) | 0 | 2 (8.3) | 1 (4.5) | 1 (5.6) | 0.800 |
| Other infection, \(n(\%)\) | 7 (9.5) | 1 (10.0) | 2 (8.3) | 3 (13.6) | 1 (5.6) | 0.848 |
| CMV and EBV = 1 | EBV = 1 | Bacteria = 1 | HSV = 1 | 0.948 |
| Other infection, \(n(\%)\) | 20 (27.0) | 1 (10.0) | 3 (12.5) | 7 (31.8) | 9 (50.0) | 0.028 |

* The patient was diagnosed with multiple myeloma (IgG type, Durie-Salmon stage I group A) 9 years before the renal biopsy, and he had no renal involvement at that time. He received 4 cycles of VTD, chemotherapy. At the time of renal biopsy, his serum IFE showed bicalon IgGκ and IgGλ. He received throughout bone marrow tests neither of the serum-free light chains, bone marrow smear, flow cytometry, and FISH; biopsy revealed restricted plasma cell disorder. He also received CT scans throughout to rule out a pathological bone fracture. Besides, his cryoglobulinemia was type III, and he had an active HBV infection. The patient was diagnosed with type I cryoglobulinemia (IgGκ) but had repeatedly negative serum/urine IFE. His bone marrow smear did not show plasma disorder and an ultrasound scan did not reveal lymphopathy. The x-ray scan of the bone did not show a bone fracture. After 42 months of follow-up, the evidence of lymphoproliferative disease was still absent. He did not have evidence of viral infection, either. So the patient was attributed to “essential” temporally despite his monoclonal cryoglobulinemia.
Characteristics of Renal Presentation

The renal manifestations were summarized in Table 3. Edema was the most common initial symptom ($n = 46$, 62%), followed by gross hematuria ($n = 8$, 11%), while still quite a lot of patients were “insidious” ($n = 16$, 21%). The median interval between diagnosis and renal disease onset was 8 (range 1–120) months. The median UTP was 4.92 (0.41–15.44) g/day, and the mean serum Alb level was 27.54 ± 6.19 g/L. Thirty-nine patients (53%) had nephrotic syndrome. Ninety-one percent ($n = 67$) of patients had microscopic hematuria, ranging from mild (26%) to severe (19%). The mean eGFR was 58.13 ± 31.96 mL/min/1.7 m², and 42% ($n = 31$) of patients had renal function worse than the CKD3b stage at diagnosis. Thirty-two percent ($n = 24$) of patients had experienced AKI around renal biopsy. Two patients had an eGFR <15 mL/min/1.7 m², but neither was on renal replacement therapy at diagnosis. Among the four groups, patients with type I

Table 2. The demographic and clinical features of various Cryo-GN

| Characteristics                  | Total ($n = 74$) | Type I ($n = 10$) | Type II ($n = 24$) | Type III ($n = 22$) | Unknown type ($n = 18$) | p value |
|----------------------------------|-----------------|------------------|-------------------|---------------------|------------------------|---------|
| Age at diagnosis, mean±SD, years | 52.9±15.0       | 55.4±12.1        | 53.6±16.3         | 50.3±16.6           | 52.9±15.0              | 0.502   |
| Female, n (%)                    | 21 (28.4)       | 2 (20.0)         | 5 (20.8)          | 6 (27.3)            | 8 (44.4)               | 0.345   |
| HTN, n (%)                       | 54 (73.0)       | 6 (60.0)         | 19 (79.2)         | 17 (77.3)           | 12 (66.7)              | 0.596   |
| DM, n (%)                        | 11 (14.9)       | 4 (40.0)         | 4 (16.7)          | 3 (13.6)            | 0                      | 0.042   |
| Anemia, n (%)                    | 60 (81.1)       | 9 (90.0)         | 22 (91.7)         | 17 (77.3)           | 12 (66.7)              | 0.178   |
| Ex-renal manifestations, n (%)   | 21 (28.4)       | 3 (30.0)         | 9 (37.5)          | 3 (13.6)            | 6 (33.3)               | 0.312   |
| Hypocomplementemia, n (%)        | 33 (44.6)       | 5 (50.0)         | 13 (54.2)         | 7 (31.8)            | 8 (44.4)               | 0.482   |
| Decreased C4 level               | 23 (31.1)       | 5 (50.0)         | 9 (37.5)          | 4 (18.2)            | 5 (27.8)               | 0.275   |
| IgG, mean±SD, g/L                | 7.47±3.72       | 7.69±4.53        | 7.35±3.73         | 8.70±3.94           | 6.01±2.51              | 0.154   |
| IgA, mean±SD, g/L                | 1.79±1.10       | 1.47±0.86        | 1.64±1.21         | 2.23±1.11           | 1.64±0.97              | 0.160   |
| IgM, mean±SD, g/L                | 1.53±1.40       | 1.42±1.43        | 1.87±1.62         | 1.27±0.64           | 1.46±1.75              | 0.537   |
| Increased RF activity, n (%)      | 18 (29.0)       | 2 (33.3)         | 9 (40.9)          | 3 (15.8)            | 4 (26.7)               | 0.359   |
| Positive ANA, n (%)              | 14 (18.9)       | 2 (20.0)         | 4 (16.7)          | 7 (31.8)            | 1 (5.6)                | 0.206   |

HTN, hypertension; DM, diabetes mellitus; RF, rheumatoid factor; ANA, antinuclear antibodies. # One patient had positive Sm antibody and U1RNP antibody with ANA 1:3,200. * One patient had a positive PCNA antibody with ANA 1:320.

Table 3. The renal features of various Cryo-GN

| Characteristics                  | Total ($n = 74$) | Type I ($n = 10$) | Type II ($n = 24$) | Type III ($n = 22$) | Unknown type ($n = 18$) | p value |
|----------------------------------|-----------------|------------------|-------------------|---------------------|------------------------|---------|
| Initial symptoms, n (%)          |                 |                  |                   |                     |                        |         |
| Edema, n (%)                     | 46 (62.2)       | 4 (40.0)         | 12 (50.0)         | 15 (68.2)           | 15 (83.3)              | 0.061   |
| Gross hematuria, n (%)           | 8 (10.8)        | 4 (40.0)         | 3 (12.5)          | 0                   | 1 (5.6)                | 0.007   |
| Insidious, n (%)                 | 16 (21.6)       | 2 (20.0)         | 7 (29.2)          | 6 (27.3)            | 1 (5.6)                | 0.635   |
| UTP, mean ± SD, g/day            | 5.95±3.88       | 6.12±3.54        | 5.06±4.56         | 6.88±4.96           | 5.94±3.89              | 0.583   |
| Alb, mean ± SD, g/L              | 27.54±6.19      | 28.25±6.62       | 27.95±7.61        | 26.84±6.07          | 27.44±4.72             | 0.917   |
| Nephrotic syndrome, n (%)         | 39 (52.7)       | 5 (50.0)         | 12 (50.0)         | 10 (45.5)           | 12 (66.7)              | 0.578   |
| Microscopic hematuria, n (%)     | 67 (90.5)       | 10 (100.0)       | 21 (95.9)         | 19 (95.9)           | 17 (98.6)              | 0.049   |
| Mild                             | 19 (25.7)       | 1 (10.0)         | 7 (29.2)          | 6 (27.3)            | 5 (27.8)               | 0.249   |
| Moderate                         | 26 (35.1)       | 3 (30.0)         | 5 (20.8)          | 10 (45.5)           | 8 (44.4)               | 0.444   |
| Severe                           | 22 (29.7)       | 6 (60.0)         | 9 (37.5)          | 3 (13.6)            | 4 (22.2)               | 0.405   |
| Scr, mean ± SD, μmol/L           | 143.6±77.64     | 140.48±53.90     | 146.59±79.53      | 145.40±65.56        | 139.31±102.12          | 0.990   |
| eGFR, mean ± SD, mL/min/1.7 m²   | 58.13±31.96     | 53.55±28.98      | 60.16±38.29       | 57.73±30.12         | 58.44±28.65            | 0.960   |
| Assumed AKI, n (%)               | 24 (32.4)       | 5 (50.0)         | 8 (33.3)          | 6 (27.3)            | 5 (27.8)               | 0.601   |
cryoglobulinemia tended to have more severe microscopic hematuria, other renal parameters were similar among the four groups.

Pathological Findings

Table 4 presents the renal pathological findings. MPGN was the predominant histologic pattern seen in 70% (n = 52) of patients, characterized by mesangial hypercellularity, capillary wall remodeling with double contours (Fig. 2a). Endocapillary proliferative glomerulonephritis accounted for 19% (n = 14), featured by endocapillary hypercellularity and leukocyte infiltration (Fig. 2b). Wire-loop/intraluminal pseudo-thrombi were observed in 49% (n = 36) of patients. Cellular/fibro-cellular crescent formation was seen in 49% (n = 36) with a mean of 8%. Global glomerulosclerosis was described in 77% (n = 57) patients with a mean of 14%, and interstitial fibrosis and tubular atrophy were described in 80% (n = 59) of patients with a mean of 20%. Immunofluorescence localized the granular immune deposits in the glomerular capillary wall and less frequently in the mesangium (Fig. 2c). The deposits were stained mostly for IgG (n = 51) and IgM (n = 58) and less frequently for IgA (n = 37). κ or λ light-chain restriction was reported in 11 patients with type I (n = 3) or II (n = 8) cryoglobulinemia, mostly κ restriction (n = 8) (online suppl. Table S1). C3 staining was observed in almost all patients (n = 68), whereas C1q staining was reported in nearly 60% of patients (n = 43). Electron microscopy analysis was performed in all patients. Organized substructures were observed in 23% (n = 17) of patients, primarily microtubes with a diameter of 10–55 nm (Fig. 2d). Granular nonorganized dense deposits were observed in most (n = 65) cases. The dense deposits were located in mesangial (n = 60), subendothelial (n = 57), subepithelial (n = 27), and intramembranous (n = 14) areas. No significant difference was observed in pathological features among the four groups.

Treatment and Clinical Outcomes

Overall, 89% (n = 66) of patients were followed up for a median of 24 (range from 1 to 123) months. Only 8 patients were lost to follow-up after discharge from the hospital. No difference in renal parameters was observed between patients with or lost to follow-up. At the end of the follow-up, 18 patients reached ESRD and 6 patients were on renal replacement therapy; the estimated median renal survival was 84 (95% confidence interval: 40–127) months, no significant difference was observed among

Table 4. The renal histopathological assessments of various Cryo-GN

| Characteristics                        | Total (n = 74) | Type I (n = 10) | Type II (n = 24) | Type III (n = 22) | Unknown type (n = 18) | p value |
|----------------------------------------|---------------|----------------|-----------------|------------------|----------------------|---------|
| Histological pattern, n (%)           |               |                |                 |                  |                      |         |
| MPGN                                   | 52 (70.3)     | 5 (50.0)       | 18 (75.0)       | 15 (68.2)        | 14 (77.9)            | 0.431   |
| EPGN                                   | 15 (20.3)     | 3 (30.0)       | 5 (20.8)        | 4 (18.2)         | 3 (16.4)             |         |
| Others                                 | 8 (10.8)      | 3 (30.0)       | 1 (4.2)         | 3 (13.6)         | 1 (5.6)              |         |
| Wire-loop/pseudo-thrombi, n (%)        | 36 (48.6)     | 6 (60.0)       | 9 (37.5)        | 14 (63.6)        | 7 (38.9)             | 0.224   |
| Crescent formation, n (%)              | 36 (48.6)     | 6 (60.0)       | 16 (66.7)       | 9 (40.9)         | 5 (27.8)             | 0.063   |
| IFTA, n (%)                            | 59 (79.7)     | 9 (90.0)       | 18 (75.0)       | 18 (81.8)        | 14 (77.8)            | 0.780   |
| Glomerulosclerosis, n (%)              | 57 (77.0)     | 7 (70.0)       | 19 (79.2)       | 19 (86.4)        | 12 (66.7)            | 0.472   |
| Immunofluorescence, n (%)              |               |                |                 |                  |                      |         |
| IgG                                    | 51 (68.9)     | 6 (60.0)       | 13 (54.2)       | 17 (777.3)       | 15 (83.3)            | 0.153   |
| IgA                                    | 37 (50.0)     | 4 (40.0)       | 11 (45.8)       | 14 (63.6)        | 8 (44.4)             | 0.489   |
| IgM                                    | 58 (78.4)     | 7 (70.0)       | 19 (79.2)       | 21 (95.5)        | 11 (61.1)            | 0.061   |
| C3                                     | 68 (91.9)     | 9 (88.9)       | 21 (87.5)       | 22 (100.0)       | 16 (88.9)            | 0.419   |
| C1q                                    | 43 (58.1)     | 4 (40.0)       | 11 (45.8)       | 16 (72.7)        | 12 (66.7)            | 0.151   |
| Organized substructure, n (%)          | 17 (23.0)     | 5 (50.0)       | 5 (20.8)        | 5 (22.7)         | 2 (11.1)             | 0.132   |
| Nonorganized dense deposits, n (%)     | 67 (90.5)     | 10 (100)       | 20 (83.3)       | 22 (100)         | 15 (83.3)            | 0.117   |
| Mesangial                              | 62 (83.7)     | 8 (80.0)       | 18 (75.0)       | 22 (100)         | 14 (77.7)            | 0.102   |
| Subendothelial                         | 59 (79.7)     | 9 (90.0)       | 17 (70.8)       | 21 (95.5)        | 13 (72.2)            | 0.245   |
| Subepithelial                          | 29 (39.1)     | 4 (40.0)       | 7 (29.2)        | 11 (50.0)        | 7 (38.9)             | 0.553   |
| Intramembranous                        | 15 (20.3)     | 1 (10.0)       | 5 (20.8)        | 8 (36.3)         | 1 (5.6)              | 0.086   |

MPGN, membranoproliferative glomerulonephritis; EPGN, endothelial proliferative glomerulonephritis; IFTA, interstitial fibrosis/tubular atrophy.
each type of Cryo-GN (Fig. 3). Seventy percent (n = 43) of patients received RAS inhibitor therapy, and 9 patients received plasmapheresis (PEX) due to acute renal deterioration. The clinical features and treatments of 5 patients with hematological malignancies and type I or II cryoglobulinemia are listed in Table 5. Seventeen patients only received supportive therapy (including blood pressure control, restriction of sodium intake, antiviral, or antibacterial therapy if necessary) because of spontaneous complete or partial remission or decline in the treatment. Compared to those with immunosuppressive therapy (steroids with or without cytotoxic agents) or clone-targeted therapy (rituximab [RTX] or bortezomib-based chemotherapy), they had a better renal function and less UTP at diagnosis. In the patients with nonmalignant type I or II cryoglobulinemia (n = 28), no significant difference was observed in baseline renal parameters and renal outcomes between the immunosuppressive group and the clone-targeted group, but the rate of eGFR decline was significantly lower in patients who received clone-target-
ed therapy compared with those who received immunosuppressive agents ($p = 0.049$). In patients with type III or undetermined cryoglobulinemia ($n = 34$), the immunosuppressive agents tended to delay renal progression, but the difference did not reach statistical significance. There was no significant difference in renal responses to immunosuppressive agents between different types of cryoglobulinemia (type I and II vs. type III and undetermined, $p = 0.535$). In a subgroup analysis, for those with hepatitis virus-related Cryo-GN, oral antiviral agents were given to the 15 patients with active viral infection (HBV = 12, HCV = 3). Among the 15 patients, eight received a com-

Table 5. Clinical and pathological features, treatments, and renal outcomes of hematological malignancy-related Cryo-GN

| No. | Type of cryoglobulinemia | Hematological malignancies | Age, years | Gender | Histological pattern | Treatments | Follow-up time, months | Alb, g/L (at diagnosis/at last follow-up) | UTP, g/day (at diagnosis/at last follow-up) | eGFR, mL/min/1.7 m² (at diagnosis/at last follow-up) |
|-----|--------------------------|---------------------------|------------|--------|----------------------|------------|----------------------|-----------------------------------------|------------------------------------------|--------------------------------------------|
| 1   | I                        | LPL                       | 60         | M      | MPGN                 | PEX + Fc   | 24                   | 31.2/38.4                              | 4.99/0.07                                | 41.19/92.72                               |
| 2   | I                        | CLL/SBCL                  | 67         | M      | MPGN                 | Zanubrutinib + RTX | 12                   | 22.6/26.8                              | 12.88/5.54                              | 27.09/12.98                               |
| 3   | II                       | POEMS                     | 51         | M      | MPGN                 | Steroids + CTX | 27                   | 34.6/40.0                              | 1.77/1.44                                | 40.81/90.05                               |
| 4   | I                        | MM                        | 57         | F      | EPGN + TMA           | PEX + BCD  | 36                   | 30.1/36.5                              | 0.58/0.00                               | 67.97/NA                                  |
| 5   | I                        | CLL/SBCL                  | 62         | M      | FGPN                 | NA         | Lost                 | 27.1/NA                                | 4.21/NA                                  | 67.97/NA                                  |

One patient with previous MM had active HBV infection and type III cryoglobulinemia, and his bone marrow did not reveal an abnormality; thus, his Cryo-GN was considered as nonmalignancies related. LPL, lymphoplasmacytic lymphoma; CLL/SBCL, chronic B lymphocyte leukemia/small B-cell lymphoma; POEMS, polynuropathy, organomegaly, endocrinopathy, monoclonal protein, and skin changes; MM, multiple myeloma; MPGN, membranoproliferative glomerulonephritis; FGPN, focal proliferative glomerulonephritis; TMA, thrombotic microangiopathy; PEX, plasmapheresis; Fc, fludarabine + cyclophosphamide; RTX, rituximab; BCD, bortezomib + cyclophosphamide + dexamethasone; Alb, albumin; UTP, proteinuria; eGFR, estimated glomerular filtration rate; RPGN, rapidly progressive glomerulonephritis; RRT, renal replacement therapy.

Table 6. Different treatments and renal outcomes of nonmalignancy-related Cryo-GN

| Supportive therapy (n = 17) | Immunosuppressive therapy (administered)* (n = 43) | Clone-targeted therapy (cumulative)* (n = 8) | p value |
|-----------------------------|----------------------------------------------------|-----------------------------------------------|---------|
| UTP at baseline, mean±SD, g/day | 4.09±3.39                                         | 6.90±4.67                                    | 6.17±3.76 | 0.081 |
| eGFR at baseline, mean±SD, mL/min/1.7 m² | 79.79±38.19                                      | 54.24±29.38                                  | 40.13±20.70 | 0.005 |
| RAS inhibitor, n (%) | 14 (22.6)                                         | 23 (37.1)                                    | 6 (9.7)    | 0.305 |
| PEX, n (%) | 0                                                 | 5 (8.1)                                      | 2 (3.2)    | 0.112 |
| Type I and II cryoglobulinemia (n = 28) | 6 (17.6)                                         | 20 (71.4)                                    | 8 (28.6)   | 0.795 |
| UTP at baseline, mean±SD, g/day | 4.63±4.16                                         | 5.55±4.37                                    | 6.17±3.76 | 0.005 |
| eGFR at baseline, mean±SD, mL/min/1.7 m² | 98.34±44.45                                      | 52.70±31.01                                  | 40.13±20.70 | 0.005 |
| Renal response, n (%) | 5 (83.3)                                         | 6 (30.0)                                     | 3 (37.5)   | 0.065 |
| ESRD, n (%) | 0                                                 | 7 (35.0)                                     | 2 (25.0)   | 0.233 |
| Decline rate of eGFR, median with range, mL/min/1.7 m²/month | 3.73 (0.27, 7.55) | 0.80 (−0.89, 12.64) | 0.22 (−1.3, 1.01) | 0.043 |
| Type III and undetermined cryoglobulinemia (n = 34) | 11 (32.4)                                         | 23 (67.6)                                    | 0         | 0.010 |
| UTP at baseline, mean±SD, g/day | 3.79±3.07                                         | 8.07±4.69                                    | 55.58±28.52 | 0.205 |
| eGFR at baseline, mean±SD, mL/min/1.7 m² | 69.66±32.06                                      | 55.58±28.52                                  | 0.714     | 0.810 |
| Renal response, n (%) | 6 (54.5)                                         | 11 (47.8)                                    | 3 (37.5)   | 0.176 |
| ESRD, n (%) | 2 (18.2)                                         | 5 (21.7)                                     | 0         | 0.176 |
| Decline rate of eGFR, median with range, mL/min/1.7 m²/month | 0.33 (−0.94, 7.81) | 0.21 (−3.56, 6.81) | 0.126     | 0.176 |
| Antiviral therapy (n = 15) | 7                                                  | 8                                              | 0         | 0.051 |
| UTP at baseline, mean±SD, g/day | 3.85±2.41                                         | 8.03±4.87                                    | 0.039     | 0.544 |
| eGFR at baseline, mean±SD, mL/min/1.7 m² | 84.67±46.48                                      | 42.07±23.39                                  | 0.573     | 0.121 |
| Renal response, n (%) | 5 (71.4)                                         | 5 (62.5)                                     | 1 (14.3)   | 0.121 |
| ESRD, n (%) | 1 (14.3)                                         | 2 (25.0)                                     | 0         | 0.121 |
| Decline rate of eGFR, median with range, mL/min/1.7 m²/month | 0.34 (−0.94, 6.05) | −0.88 (−2.3, 0.83) | 0.121     | 0.121 |

* Six patients with type I or II cryoglobulinemia received steroids combined with cytotoxic agents therapy as first-line therapy, but none of them had a renal response, so they turned to clone-targeted therapy sequentially. Two patients received clone-targeted therapy and PEX as first-line therapy, and both achieved renal remission.
bination of immunosuppressive therapy. The patients who received antiviral agents alone had a better renal function and less UTP at diagnosis. But the renal outcomes were similar between the two groups, and the immunosuppressive therapy tended to retard the renal progression (Table 6).

By multivariate Cox analysis, only eGFR <45 mL/min/1.73 m² was the independent factor associated with ESRD at the last follow-up (HR 4.21, 95% confidence interval, 1.54–11.503, p = 0.005). The reports of treatment-related side effects were limited. Two patients who received immunosuppressive therapy had severe pneumonia and rehospitalization during follow-up, one with a fungal infection and the other with a bacterial infection.

Discussion

To our knowledge, our single-center study included the largest number of patients with Cryo-GN in China so far [12–14]. In our research, we comprehensively analyzed the etiological and clinicopathological spectrum of various Cryo-GN. In the absence of autoimmune disorders, it turned out that monoclonal gammopathy was prominent in type I or II cryoglobulinemia, and more patients with type III or undetermined cryoglobulinemia were classified as “essential.” In contrast to the situation in Western countries [4, 15], HBV infection was far more common in Chinese patients with cryoglobulinemia than with HCV infection. Although the etiology varied between different types of cryoglobulinemia, the clinical manifestations were similar. Hematuria, UTP, hypertension, anemia, and chronic renal insufficiency were the most common features. Besides, about 50% of patients presented with nephrotic syndrome, and 30% experienced AKI. Hypocomplementemia, serum-positive RF activity, and skin lesions were reported in around 30% of patients. These findings were consistent with the previously reported case series [5, 7, 16, 17].

According to the new consensus of monoclonal gammopathy of renal significance, type II Cryo-GN was classified as an MGRS-related disease [16]. But the association of type II cryoglobulinemia with monoclonal gammopathy was seldom reported in the published medical literature [4, 7, 18, 19]. And the light-chain restriction of renal tissue was challenging to confirm owing to the polyclonal Ig component of type II cryoglobulins [7]. In one series of 20 patients with noninfectious type II Cryo-GN, monoclonal gammopathy was reported in 17 patients, yet without reference to serum/urine IFE [20]. Whether the isotype of serum/urine monoclonal Ig was consistent with the cryoprecipitate (IgMκ) was unknown. In a case report by Flavell et al. [17], 4 patients with noninfectious mixed (type II or III) Cryo-GN and IgM-κ monoclonal gammopathy were recorded. However, no one showed light-chain restriction on paraffin-immunofluorescent microscopy. Three of the 4 patients had primary Sjögren syndrome [20]. In our report, the isotype of serum monoclonal Ig was consistent with monoclonal cryoprecipitate in 11 patients with type II cryoglobulinemia, and seven among whom showed renal light-chain restriction on immunofluorescent microscopy. Our findings provide a strong association between monoclonal gammopathy and type II Cryo-GN. However, it has been known that HCV infection-driven B-cell activation and proliferation can lead to both mixed cryoglobulinemia and monoclonal gammopathy [21]. Our study found four of the eleven type II cryglobulinemic patients with active HBV infection (n = 2) or HCV infection (n = 2) and monoclonal gammopathy, among whom two showed light-chain restriction on immunofluorescent microscopy. This finding reminds us that when these two diseases are associated with chronic hepatitis virus infection, it must be carefully judged whether the relationship between them is accompanying (both secondary to HBV or HCV infection) or causal (mixed cryoglobulinemia caused by monoclonal gammopathy).

Proliferative glomerulonephritis, especially MPGN, accounted for the most of histopathological types of Cryo-GN. By light microscopy, there are some characteristics that can help distinguish proliferative Cryo-GN from primary proliferative glomerulonephritis. Eosinophilic and periodic acid-Schiff stain (PAS)-strongly positive deposits in the subendothelial sites and within capillary lumens (so-called pseudo-thrombi) often appear in Cryo-GN [22]. By immunofluorescent microscopy, there are granular immune deposits in the mesangium and capillary wall, which often sketch a petal-like glomerular outline in the type of MPGN. There is prominent IgM and C3, often with the clonal bias of κ light chain versus λ, with similar or lesser amounts of IgG or C1q [10, 22]. By electron microscopy, the deposits in mesangial, subendothelial, and capillary lumens are often seen. The ultrastructural appearance varied from the most classical appearance of annular, curved, and paired short microtubules to randomly disposed microtubular structures to microtubular groupings forming paracrystalline arrays [23]. There was no specific relationship between the structural appearance of the deposits and the type of cryoglobulinemia. However, not all electron microscopic
specimens of patients can detect the above substructures. The diagnosis of Cryo-GN cannot be denied simply because no substructures have been found [7, 20, 24].

The optimal treatment recommendations based on high-quality evidence for Cryo-GN are still limited. The treatment measures are various and highly individualized according to associated disease and severity for different cryoglobulinemic types and different underlying disorders [25]. In our study, immunosuppressive therapy, including glucocorticoids and cytotoxic agents, was given to most nonmalignancy-related patients, but the renal responses were poor, and the renal outcomes were unfavorable. For patients with type I or II Cryo-GN, clone-targeted treatment, including RTX aiming at B lymphocytes and bortezomib aiming at plasma cells, could retard the renal deterioration compared to the immunosuppressive therapy. The use of RTX in monoclonal or mixed cryoglobulinemia has been reported in several reports and achieved a relatively high response rate [5, 7, 26]. But, it could also lead to disease flare in all types of cryoglobulinemic vasculitis [27]. Bortezomib has been reported for successfully treating non-IgM type I cryoglobulinemic vasculitis, even in patients who failed to respond to RTX [28, 29]. However, in our study, we tried to use bortezomib-based chemotherapy after RTX failure in 2 patients with IgG monotype of monoclonal gammopathy and cryoglobulinemia. Still, neither showed a response to the proteasome inhibitor. Since RTX and bortezomib were not covered by health insurance for treating cryoglobulinemia in China, their application was limited for economic issues. A response to PEX in mixed cryoglobulinemia is seen in 70%–80% of patients [30], and PEX is a viable therapeutic option with severe disease manifestations, especially in cases with AKI, to prevent irreversible lesions [31]. In our study, 5 out of the 9 patients receiving plasma exchange had a renal response. Considering that PEX does not inhibit the generation of new cryoglobulins, it is rational to combine with other immunosuppressive therapies to reduce the production of cryoglobulins. For patients with hepatitis viral-related Cryo-GN, the effect of antiviral agents was confirmed by several studies, especially in mild or moderate cases. However, in severe cases, immunosuppressive therapy based on efficient antiviral therapy was still recommended [19, 32–34]. In our study, the effect of immunosuppressive agents in preventing renal deterioration based on efficient antiviral treatment was also being proven.

The present study has limitations. The retrospective data collection accounted for potential biases, notably in assessing extra-renal manifestations, cumulative drug dose, and treatment-related side effects. The lack of cryoglobulins quantitative tests, both at diagnosis and during follow-up, limited the analysis of treatment effects. The relatively short follow-up period limited the accuracy of the disease relapse and prognosis analysis.

**Conclusion**

In conclusion, our study describes the spectrum and outcome of patients with various Cryo-GN in China. Although the etiology is diverse, the clinical and pathological manifestations are similar. Our data strongly support the association between monoclonal gammopathy and type II Cryo-GN. However, the relationships between hepatitis viral infection, monoclonal gammopathy, and cryoglobulinemia are sometimes still confounding. Even though widely used, the renal responsive rate of immunosuppressant therapy is still suboptimal. The clone-targeted treatment shows promising effects in patients with type I or II Cryo-GN.

**Statement of Ethics**

Written informed consent was obtained from each patient for demographic and clinical data, renal issues, blood samples, and urine samples. The research complied with the declaration of Helsinki and was approved by the Ethics Committee of Peking University First Hospital (approval number 2017 [1280]).

**Conflict of Interest Statement**

The authors have no conflicts of interest to declare.

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**Author Contributions**

Xin Zhang analyzed the data, interpreted the results, and wrote the manuscript. Xin Zhang, Xiao-juan Yu, and Zi-hao Yong contributed to data collection. Chong-wen An contributed to the cryoglobulins detection. Xiao-juan Yu and Su-xia Wang reviewed the renal biopsies independently. Fu-de Zhou, and Ming-hui Zhao contributed....
contributed to the revision of the manuscript. Xio-juan Yu and Chong-wen An are the co-corresponding authors and were involved in the study design, data interpretation, and manuscript revision. All authors participated in the design of the study, and read and approved the final manuscript.

**Data Availability Statement**

All data generated or analyzed during this study are included in this published article and its online supplementary material. Further inquiries can be directed to the corresponding author.