Immunotactoid glomerulopathy: clinicopathologic and proteomic study

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Abstract

Background. Immunotactoid glomerulopathy (ITG) is a rare glomerular disease. Here, we report the largest clinicopathologic series of ITG and define its proteomic profile.

Methods. The characteristics of 16 ITG patients who were identified from our pathology archives are provided between 1993 and 2011. We also performed laser microdissection and mass spectrometry (LMD/MS) in three cases.

Results. Presentation included proteinuria (100%), nephrotic syndrome (69%), renal insufficiency (50%) and microhematuria (80%). Hypocomplementemia was present in 46% and a serum M-spike in 63%. Hematologic malignancy was present in 38%, including chronic lymphocytic leukemia in 19%, lymphoplasmacytic lymphoma in 13% and multiple myeloma in 13%. The pattern of glomerular injury was membranoproliferative (56%), membranous (31%) or proliferative (13%) glomerulonephritis. The microtubular deposits were immunoglobulin light chain restricted in 69% and had a mean diameter of 31 nm (range 17–52). During an average of 48 months of follow-up for 12 patients, 50% had remission, 33% had persistent renal dysfunction and 17% progressed to end-stage renal disease. Proteomic analysis by LMD/MS revealed the presence of immunoglobulins, monotypic light chains, complement factors of the classical and terminal pathway and small amount of serum amyloid P-component.

Conclusions. Hematologic malignancy, particularly lymphoma, is not uncommon in ITG. ITG appears to have a better prognosis than other paraprotein-related renal lesions, with a half of patients expected to recover kidney function with immunosuppressive therapy or chemotherapy. The proteomic profile of ITG is consistent with deposition of monotypic immunoglobulins and activation of the classical and terminal pathway of complement.

Keywords: dysproteinemia; glomerulonephritis; immunotactoid glomerulopathy; lymphoma

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Introduction

Immunotactoid (microtubular) glomerulopathy (ITG) is a term introduced by Schwartz and Lewis [1, 2] to describe a glomerular disease characterized by the presence of Congo-red-negative organized glomerular deposits that stain for IgG and complement by immunofluorescence (IF). These authors recommend limiting the diagnosis of ITG to patients without underlying hematologic disorders, cryoglobulinemia or systemic lupus erythematosus [2]. In their view, ITG includes cases that show Congo-red negative, randomly-oriented fibrils (i.e., fibrillar glomerulonephritis) and cases that show larger microtubular structures [2]. However, several studies from other groups have found that the characteristics of fibrillar glomerulonephritis are distinctly different from those of ITG: patients with fibrillar glomerulonephritis are less likely to have hypocomplementemia or underlying dysproteinemia than ITG patients, and the glomerular deposits are often polyclonal [3–7]. Therefore, most investigators currently advocate separating ITG from fibrillar glomerulonephritis [3–6]. The majority of published series on ITG have included patients with underlying hematologic malignancy (lymphomas and/or plasma cell dyscrasias), but excluded patients with systemic lupus erythematosus or cryoglobulinemia [3–5], although few patients included in some of these series had a transiently positive test for serum cryoglobulin [3, 4].

When cryoglobulinemia and systemic lupus erythematosus are excluded, ITG is a very rare glomerular disease encountered in 0.06% of native kidney biopsies [3]. Patients with ITG typically present with proteinuria (usually nephrotic), hematuria, renal insufficiency and hypertension. ITG is frequently associated with hypocomplementemia, monoclonal gammapathy and lymphoproliferative disorders. Data on prognosis and appropriate therapy are limited due to the lack of large studies. ITG can recur in the transplanted kidney. The disease may histologically exhibit membranoproliferative, diffuse proliferative or membranous patterns of glomerular injury. In most cases, the deposits contain IgG and C3 and exhibit light chain restriction by IF [3, 4]. Ultrastructurally, the glomerular microtubules measure 9–45 nm in width and are located predominantly in the subepithelial space and subendothelial space [4]. The pathogenesis of ITG is currently unknown. The unique microtubular organization is likely influenced by abnormalities in the structure of the monoclonal protein, its physicochemical properties and its tissue affinity.

In the current report, we retrospectively examined 16 cases of ITG diagnosed on renal biopsy at a large renal biopsy referral center from 1993 to 2011, which represent the largest series to date. Our aim is to define the disease’s demographic, hematologic and renal characteristics, histologic findings, treatment and outcome. We also performed laser microdissection and mass spectrometry (LMD/MS) to define the proteomic profile of ITG, which has not been established in previous studies.

Materials and methods

Twenty patients with ITG were identified from the archives of the Renal Pathology Laboratory of Mayo Clinic, Rochester, between 1993 and 2011. Four of these patients were excluded from the study because of the lack of glomeruli for IF. All remaining 16 cases fulfilled the following diagnostic criteria of ITG: glomerular deposition of microtubules that (i) had distinct hollow centers at magnification of >30000, (ii) arranged at least focally in parallel arrays and (iii) stained with antisera to immunoglobulins by IF. Biopsies with clinicopathologic diagnosis of cryoglobulinemic glomerulonephritis or lupus nephritis were excluded.

All renal biopsies were processed according to standard techniques for LM, IF and electron microscopy (EM). For LM, all cases were stained with hematoxylin and eosin, periodic acid Schiff (PAS), Masson’s trichrome and Jones methenamine silver. IF was performed on 3-µm cryostat sections using polyclonal fluorescein isothiocyanate (FITC)-conjugated antibodies to IgG, IgM, IgA, C3, C1q, kappa, lambda, fibrinogen and albumin (Dako Corporation, Carpinteria, CA, USA). Determination of the IgG subclass, done in seven cases, was performed on 3-µm cryostat sections using monoclonal FITC-conjugated antibodies to IgG1, IgG2, IgG3 and IgG4 (Sigma-Aldrich Corp., St. Louis, MO, USA). LMD/MS was performed on three cases in which glomeruli were seen in the residual paraffin-embedded tissue. The methods for LMD/MS have previously been described in detail [8, 9].

Demographic information, presenting renal clinical and laboratory findings and hematologic clinical and laboratory findings (including findings on bone marrow biopsy), treatment and follow-up, were obtained from patients’ medical records. Quantification of proteinuria was performed by 24-h collection (11 patients) or by the spot urine protein-to-creatinine ratio when 24-h urine collection was not performed (3 patients). The following clinical definitions were used: nephrotic-range proteinuria (NRP): ≥3.0 g/day; hypoalbuminemia: serum albumin <3.5 g/dL; renal insufficiency: serum creatinine >1.2 mg/dL and nephrotic syndrome: NRP with hypoalbuminemia and peripheral edema. Tubular atrophy and interstitial fibrosis were graded on a semiquantitative scale based on an estimate of the percentage of the renal cortex affected and recorded as: 0 (none), 1–25% (mild), 26–50% (moderate) or >50% (severe). For the purpose of outcome analysis, the following definitions were used: (i) complete remission (CR): remission of proteinuria to <0.5 g/day with normal renal function; (ii) partial remission (PR): reduction in proteinuria by at least 50% and to <2 g/day with stable renal function (no more than a 20% increase in serum creatinine); (iii) persistent renal dysfunction (PRD): failure to meet criteria for either CR or PR but not reaching end-stage renal disease (ESRD), including patients with unremitting proteinuria or progressive chronic kidney disease and (iv) ESRD: requiring renal replacement therapy or undergoing preemptive transplant.

The study was approved by the Institutional Review Board of Mayo Clinic Foundation.

Results

Clinical features

The clinical features are summarized in Table 1. The cohort consisted of eight males and eight females, all Caucasians, with a median age of 61 years (range 41–80 years). At the time of diagnosis, eight (50%) patients had renal insufficiency. Median serum creatinine for the entire cohort was 1.5 mg/dL (range 0.8–6.2 mg/dL). All patients had proteinuria, which was within the nephrotic range in 12 of the 14 (86%) patients in whom it was quantitated. In the 11 patients with 24-h urine protein collection, proteinuria ranged from 1.2 to 13.4 g/day (median 6 g/day). In the remaining two patients, proteinuria was 600 and 487 mg/dL by protein to osmolarity ratio. Hypoalbuminemia and microhematuria were each present in 12 of the 15 (80%) patients with available data, but gross hematuria was not present in any patient. Peripheral edema was present in 13 (81%) patients. Full nephrotic syndrome was present in 9 of the 13 (69%) patients with
available data. Testing for serum complement (C), performed in 13 patients, showed normal C3 and C4 in 7 (54%), low C3 and C4 in 4 (31%), low C3 and normal C4 in 1 (8%) and normal C3 and low C4 in 1 (8%). Serum cryoglobulin was negative in all 12 patients tested. Antinuclear antibody, tested in 14 patients, was negative in 11 and weakly positive in 3. Hepatitis C antibody was negative in all 11 patients tested and antineutrophil cytoplasmic antibody was negative in all 12 patients tested. Co-existent conditions included diabetes (2 patients), psoriasis (1), melanoma (1) and alcoholic cirrhosis (1). One patient (#16) who had negative serum cryoglobulin developed a skin rash which, on biopsy, showed a dermal vasculopathy/vasculitis with negative IF staining for IgG, IgA and IgM. None of the patients had arthralgia, arthritis, skin ulcers, Raynaud’s phenomenon or peripheral neuropathy (systemic features of cryoglobulinemia and cryocryoglobulinemia).

An M-spike was identified on serum protein electrophoresis/immunofixation (SPEP/SIF) in 10 of the 16 (63%) patients, which was IgG lambda in 5, IgG kappa in 3, IgA kappa in 1 and IgG kappa plus IgA kappa in 1 (Table 1). The circulating paraprotein matched that detected in glomeruli by IF in 7 of these 10 patients (# 4, 5, 6, 7, 8, 13, 14; Tables 1 and 2). An M-spike was detected on urine protein electrophoresis/immunofixation (UPEP/UIF) in 8 of the 15 (53%) patients tested and was IgG lambda in 2, lambda in 2, IgG kappa in 2, IgA kappa in 1 and kappa plus lambda (biclonal) in 1. Serum free light chain (FLC) ratio was abnormal in only 2 of the 10 (20%) patients tested. Bone marrow biopsy, performed in nine patients, was negative for plasma cell dyscrasia or lymphoma in 3, showed <10% light chain-restricted plasmacytosis in 4 (one on which also showed lymphoplasmacytic lymphoma, LPL) and >10% light chain-restricted plasmacytosis in 2 (one of which also showed LPL). Overall, an underlying hematologic malignancy was present in 6 (38%) patients, including small B-cell lymphoma in 5 (small lymphocytic lymphoma/chronic lymphocytic leukemia, CLL, in 3 and LPL in 2) and myeloma in 2 (one of whom had a concurrent LPL). The hematologic malignancy was discovered 1.8–6 years before ITG diagnosis in three patients and concomitantly with the ITG diagnosis in the remaining three patients. Two of the three patients with CLL had chronic anemia. One patient with LPL (#5) developed hemoptysis and dyspnea (due to lung involvement by lymphoma), bilateral axillary lymphadenopathy and left cervical adenopathy. The other patient with LPL (#14) had POEMS syndrome (Polyneuropathy, Organomegaly, Endocrinopathy, Monoclonal protein, Skin changes) and inguinal lymphadenopathy. Interestingly, in this patient, an inguinal lymph node biopsy showed AL-κ amyloid in addition to LPL, whereas the kidney biopsy showed IgGκ-ITG without evidence of renal amyloid.

No follow-up was available in three patients and one patient died of sepsis shortly after biopsy. The mean duration of follow-up for the remaining 12 patients was 48 months (median 30, range 10–182). On follow-up, 1 (8%) patient had CR, 5 (42%) had PR, 4 (33%) had PRD and 2 (17%) progressed to ESRD. One of the two patients with ESRD died of unknown cause. Proteinuria decreased to <2 g/day in 6 of these 12 (50%) patients.

Of the 12 patients with follow-up, two were not treated with immunomodulatory (IM) therapy, both of whom progressed to ESRD. The remaining 10 patients received IM therapy. IM therapy consisted of steroids alone in 2, of whom 1 had PR and 1 had PRD; and steroids with one or more additional IM agents in 8, of whom 1 had CR, 4 had PR and 3 had PRD. Rituximab was used in combination with other IM agents in two patients, one had CR and one had PR. One patient with myeloma was treated with stem cell transplant; he had PRD (Table 1). Patient #1 progressed to ESRD 13 years post-biopsy. After 1 month of hemodialysis, he received a kidney from his daughter. His maintenance immunosuppressive regimen consisted of prednisone, cyclosporine and mycophenolate mofetil. An allograft biopsy performed 10 months post-transplant for worsening creatinine, proteinuria and hematuria revealed recurrent ITG. Treatment with cyclophosphamide for 10 weeks and a higher prednisone dose lead to a decease in serum creatinine to baseline and stabilization of proteinuria. However, at last follow-up 3 months following discontinuation of cyclophosphamide, proteinuria increased from 3.3 to 6.8 g/day and serum creatinine from 1.4 to 1.7 mg/dL. A repeat allograft biopsy performed 16 months post-transplantation showed persistent disease activity. This patient (who had polyclonal deposits in the kidney) never developed clinical evidence of plasma cell dyscrasia. At the time of second transplant biopsy, he had negative SPEP/SIF and normal serum FLC ratio.

Pathologic findings

Sampling for light microscopy (LM) included a median of 16 glomeruli (range 1–37 glomeruli). A median of 6% of glomeruli were globally sclerotic (Table 2). The most common histologic pattern, seen in 9 (56%) patients, was membranoproliferative glomerulonephritis (MPGN) characterized by segmental or global double-contoured glomerular capillary walls with cellular interposition and mesangial expansion by variable degrees of increased mesangial cell number, sclerosis and immune deposition (Figure 1A and B). Five of these nine (56%) cases also showed segmental membranous features with segmental spike formation on silver stain, and 2 (22%) showed massive subendothelial deposits. The second most common pattern, seen in 5 (31%) cases, was global membranous glomerulonephritis (MGN) with global glomerular basement membrane thickening and spike formation, but without duplication (Figure 1C). Three of these five cases also showed variable degrees of mesangial expansion and hypercellularity. The least common pattern, seen in only 2 (13%) cases, was endocapillary proliferative glomerulonephritis (EPGN) characterized by endocapillary hypercellularity and leukocyte infiltration causing luminal occlusion, without duplication of the glomerular basement membranes. Both of these cases also showed mild to moderate mesangial hypercellularity. The glomerular deposits, when seen by LM, were glassy and stained eosinophilic on H&E, weakly PAS positive, silver negative and trichrome blue or grey (Figure 1).
<table>
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<th>Patient #</th>
<th>Age/sex/race</th>
<th>Proteinuria (g/24)</th>
<th>eGFR</th>
<th>Hematologic conditions</th>
<th>SPEP/SIF</th>
<th>UPEP/UIF</th>
<th>FLC ratio</th>
<th>BM biopsy</th>
<th>Serum complement</th>
<th>Serum cryoglobulin</th>
<th>Duration of F/U (in months)</th>
<th>IM therapy</th>
<th>Scr at last F/U (mg/dL)</th>
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<td>Negative</td>
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<td>ND</td>
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<td>31</td>
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<td>IgGκ</td>
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<td>5-10% κ-restricted plasma cells</td>
<td>Normal C3 and C4</td>
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<td>20% κ-restricted plasma cells</td>
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<td>65</td>
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<td>IgGκ</td>
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<td>Normal C3 and C4</td>
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<td>ESRD</td>
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<td>IgGκ</td>
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<td>3% κ-restricted plasma cells and lymphoma</td>
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<td>2% κ-restricted plasma cells</td>
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<td>Negative</td>
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<td>ND</td>
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<td>Negative</td>
<td>ND</td>
<td>Low C3 and C4</td>
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<td>54</td>
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<td>Negative</td>
<td>NA</td>
<td>ND</td>
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<td>Died</td>
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<td>11 (by spot ratio)</td>
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<td>20</td>
<td>20</td>
<td>None</td>
<td>IgGκ</td>
<td>κ and λ</td>
<td>Normal</td>
<td>ND</td>
<td>ND</td>
<td>16</td>
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<td>7</td>
<td>7</td>
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<td>IgGκ</td>
<td>IgGκ</td>
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<td>None</td>
<td>IgAx/ IgGκ</td>
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<td>Normal</td>
<td>Normal C3 and low C4</td>
<td>Negative</td>
<td>No F/U</td>
<td>Died shortly after biopsy</td>
<td>Died</td>
</tr>
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</table>

BM, bone marrow; CLL, chronic lymphocytic leukemia; CR, complete remission; CYT, cyclophosphamide; eGFR, estimated glomerular filtration rate; F/U, follow-up; IM, immunomodulatory; MMF, mycophenolate mofetil; LPL, lymphoplasmacytic lymphoma; NA, not available; ND, not done; NS, nephrotic syndrome; PBSC, peripheral blood stem cell transplant; PLX, plasmapheresis; PR, partial remission; PRD, persistent renal dysfunction; Scr, serum creatinine.
Focal cellular crescents were seen in only 2 (13%) cases, while fibrinoid necrosis was not seen in any case. Twelve (80%) cases showed interstitial inflammation, which was predominantly focal. All three patients with CLL had direct focal interstitial involvement by CLL (confirmed by positive immunohistochemical staining of neoplastic lymphocytes for CD20, CD5 and CD23), while none of these two patients with LPL had renal involvement by lymphoma (Figure 1D). The degree of tubular atrophy and interstitial fibrosis ranged from absent (3 cases) to mild (11 cases) to moderate (2 cases). Arteriosclerosis ranged from absent (5 cases) to mild (6 cases) to moderate (3 cases) to severe (2 cases).

On IF, all but 2 (88%) cases showed glomerular positivity for IgG with a mean intensity of 2.3+, including two cases that showed glomerular positivity for IgM of a similar intensity (2+) (Table 2; Figure 2). The two exceptions were case #3 that showed intense (3+) staining for IgM with negative IgG and IgA, and case #15 that showed moderate staining for IgM and IgA with negative IgG. In a similar distribution to the immunoglobulin deposits, glomerular deposition of C3 was detected in 15 cases (94%; mean intensity 2.2+) and C1q in 10 (63%; mean intensity 1.7+) cases. The deposits exhibited light chain restriction in 11 (69%) cases including staining for lambda only in 6 and for kappa only in 5 cases, whereas the remaining 5 cases (31%) stained for both kappa and lambda (Figure 2). IF staining for IgG subtypes, IgG1, IgG2, IgG3 and IgG4 was performed in seven cases. The deposits were monotypic in six cases, staining for IgG1 only in four, IgG2 only in one and IgG4 only in one, whereas in the remaining case they were polytypic, staining intensely for IgG4 with weaker staining for IgG1 and IgG3.

By definition, the glomerular deposits on EM were composed of microtubules that had distinct hollow centers and were arranged at least focally in parallel arrays (Figure 3). The mean microtubule diameter was 31 nm.
Fig. 1. Light microscopic findings. The biopsy from patient #4 showed a MPGN pattern of injury with mild mesangial hypercellularity, global duplication of the glomerular basement membranes, and global large mesangial and subendothelial silver-negative immune deposits (A, ×200, B, ×400). The biopsy from patient #13 showed a membranous pattern of injury with global thickening of glomerular basement membranes by silver-negative subepithelial and intramembranous immune deposits associated with silver-positive glomerular basement membrane spikes (C, ×600). In addition to ITG, the biopsy from patient #11 showed interstitial involvement by CLL as shown in D (PAS, ×100), which exhibits a patchy interstitial infiltration by a dense monotonous population of neoplastic lymphocytes that stained positive for CD20, CD5 and CD23, with negative CD3 (not shown).

Fig. 2. Immunofluorescence findings. Immunofluorescent microscopy in patient #12 showed bright smudgy mesangial and glomerular capillary wall positivity for IgG (A, ×400) and lambda (B, ×400), with negative staining for kappa (C, ×400). The interstitial CLL cells in this patient also stained brightly for IgG (shown in D, ×200) and lambda with negative kappa (not shown).
The microtubular deposits were seen in the mesangium, in the subepithelial space and in the subendothelial space in 12 cases (75%) each. The cases with MPGN and EPGN patterns showed subendothelial deposits with or without segmental mesangial or subepithelial deposits, whereas those with the MGN pattern showed global subepithelial deposits and segmental mesangial deposits. In two cases with an MPGN pattern, there were massive mesangial and subendothelial deposits that occluded the peripheral capillary loops. None of the cases showed microtubular deposits in tubular basement membranes, interstitium or vessel walls. The degree of podocyte foot process effacement was marked in most cases. No extracellular or intracellular immunoglobulin crystals were seen in glomeruli or in the interstitium.

**Laser microdissection and mass spectrometry-based proteomic analysis**

We performed LMD/MS-based proteomic analysis to determine the glomerular proteomic profile in three cases (Figures 4 and 5). Two cases (#4 and 8) showed large numbers of peptide spectra for Ig gamma-1 chain constant region and Ig lambda-1 chain constant region, while the third case (#15) showed large numbers of peptide spectra for Ig alpha-1 chain constant region, Ig kappa-1 chain constant region, and smaller spectra for Ig mu chain constant region, Ig gamma-3 constant region and Ig lambda-1 chain constant region (Figure 5A). The Ig profile in these cases was consistent with the findings on IF. All three cases showed accumulation of complement factors of the classical and terminal pathway (Figure 5B). Thus, all cases showed peptide spectra for C3 and C4, and variable spectra for C1q, C5, C8 and C9. Surprisingly, all three cases showed peptides associated with amyloidosis such as serum amyloid P-component (SAP), apolipoprotein E and clusterin (Figure 5C).

**Discussion**

Analysis of the literature on the clinico-pathologic characteristics and outcome of ITG, a morphologically distinctive glomerular disease, is hampered by the lack of large studies, the different definitions for the disease used by different investigators and the aggregation of fibrillary glomerulonephritis with ITG by some investigators [2, 10]. ITG most frequently affects older patients. The average age at diagnosis in our series was 60 years which is similar to patients with AL amyloidosis [11] but higher than that for fibrillary glomerulonephritis [7]. There was no sex predilection in our series with a male:female ratio of 1. Underlying hematologic malignancy is frequent in ITG as it was present in 38% of our patients. In contrast to AL amyloidosis and Randall-type monoclonal immunoglobulin deposition disease in which the most common underlying hematologic malignancy is myeloma, in ITG the most common underlying malignancy is CLL. CLL infiltration into the renal interstitium is common in these cases; it was present all three patients with CLL in our cohort and in 3 of 6 CLL patients in the study by Bridoux et al. [4]. Therefore, ITG should be considered in the differential diagnosis of proteinuria in patients with CLL. Kidney biopsy with ultrastructural examination is strongly recommend in this clinical setting as the differential diagnosis is wide and includes MPGN, membranous glomerulopathy, ITG, cryoglobulinemic glomerulonephritis and proliferative glomerulonephritis with monoclonal IgG deposits [12, 13].

An M-spike was detected by SPEP/SIF in close to two-thirds of our patients with ITG. In one previous study of
ITG, a circulating paraprotein was detected by immuno-blotting in seven patients in whom SPEP/SIF was negative [4]. For comparison, M-spike was detected by SPEP/SIF in only 16% of 61 patients with fibrillary glomerulonephritis from our medical center [7]. Of note, serum FLC assay appears to be an insensitive technique to detect paraproteinemia in ITG as it was normal in six patients who had an M-spike on SPEP/SIF in our study. In contrast, serum FLC assay is abnormal in all patients with monoclonal immunoglobulin deposition disease [14] and in 78% of patients with AL amyloidosis [15]. Despite the high incidence of paraproteinemia in ITG, myeloma is uncommon, being present in only 13% of our patients.

The prognosis of ITG appears to be better than fibrillary glomerulonephritis. Only 17% of patients with ITG in our study progressed to ESRD after a mean follow-up of 48 months. In contrast, 44% of 61 patients with fibrillary glomerulonephritis from our medical center progressed to ESRD after a mean of 52 months of follow-up [7]. Therapy directed against the underlying lymphoma usually leads to remission of nephrotic syndrome. In our series, ITG remission occurred in all three patients with underlying lymphoma in whom follow-up was available. Similarly, remission of nephrotic syndrome was achieved in 5 of 7 ITG patients in the series by Bridoux et al. [4] after successful treatment of lymphoma, although recurrence of nephrotic syndrome followed by lymphoma recurrence occurred after therapy withdrawal. Due to the lack of large studies, the features associated with poor renal outcome in ITG are still unknown. A multicenter study is needed to determine the optimal therapeutic regimen, outcome and prognostic indicators.

Several morphologic patterns of glomerular injury have been described in patients with ITG, including MGN, MPGN and EPGN. In one study, atypical MGN (accompanied by mesangial expansion and hypercellularity) was the most common pattern, encountered in 71% of patients, whereas an MPGN pattern was seen in the remaining 29% of patients [4]. In our experience, the MPGN pattern was the most frequent, seen in 56% of patients (56% of whom also had segmental membranous features), followed by the MGN pattern (31%). If ITG typically exhibits an intense glomerular positivity for IgG with or without weaker positivity for IgM or IgA. However, IgG deposition is not

Fig. 5. Mass spectrometry-based proteomic analysis of glomeruli in ITG. Immunoglobulin (A), complement-related (B) and amyloid related (C) proteins identified in three cases (4, 8 and 15) are shown. The numbers reflect the number of peptide spectra identified for each protein. The probability score reflects predicted accuracy of protein identification.
always seen by IF; it was absent in two patients in our series in whom the deposits stained for IgM (two cases) and IgA (one case). There have been also rare previously reported cases of ITG in which the deposits stained predominantly for IgA [5, 16, 17]. The glomerular deposits in ITG in most cases are monoclonal and monotypic. IF staining for IgG subtypes, performed in our study and three prior studies, revealed monotypic IgG1 deposition in 61% (14 of 23) of cases tested [3, 4, 18]. Monotypic IgG3 deposits were present in only 9% of these cases, which is in sharp contrast to proliferative glomerulonephritis with monoclonal IgG deposits, a far more common disease, in which monotypic IgG3 deposits are encountered in 66% of cases [18, 19].

Microtubular deposition in ITG is generally limited to glomeruli, although one reported patient who complained of severe mononeuropathy multiplex had perineural microtubular deposits similar to those seen in glomeruli [4]. One patient in our study and two previously reported patients had cutaneous vasculitis in the absence of cryoglobulinemia [4, 20]. No immunoglobulin deposition was detected in the skin in 2 of these 3 patients, whereas in the remaining patient large immune deposits were detected by IF in and around small vessels, but no microtubular deposits were seen by EM [20].

LMD/MS-based proteomic studies confirmed the presence of Ig’s and monotypic light chains. Furthermore, the studies showed activation of the classical pathway of complement as C3 and C4 were found in all cases, in addition to components of the terminal pathway. An interesting finding was the presence of SAP and two other amyloid-associated serum proteins, apolipoprotein E and clusterin, in all three cases of ITG. SAP is found in all types of amyloid. It was also detected by gold immunoelectron microscopy in the fibrils of fibrillary glomerulonephritis, co-localizing with polyclonal IgG on the same fibril [21]. Of note, the spectra values of SAP in ITG were lower than that typically seen in amyloidosis. In addition, marked complement activation via the classical and terminal pathway that we noted in ITG is not seen in AL amyloidosis, as Ig light chains alone likely do not activate the classical complement pathway.

None of our patients had systemic features of cryoglobulinemia (such as arthralgia, arthritis, skin ulcers, Raynaud’s phenomenon or peripheral neuropathy). In this report, we excluded cases that showed histological features suggestive of cryoglobulinemic glomerulonephritis (such as intracapillary protein thrombi, numerous intracapillary infiltrating macrophages, intracellular phagocytosed deposits or non-organized deposits in addition to the microtubular deposits by EM). Serum cryoglobulin was negative in 12 patients but was not tested in 4 patients in whom cryoglobulinemia was not suspected clinically or histologically. The single patient with skin vasculitis had a negative serum cryoglobulin testing and no other features of cryoglobulinemia. However, because of the known insensitivity of the serum cryoglobulin test and histological overlap between cryoglobulinemic glomerulonephritis and ITG, we cannot exclude occult cryoglobulinemia in our patients. Occult cryoglobulinemia has been proposed by some investigators as a possible pathogenetic basis for ITG and fibrillary glomerulonephritis [4, 22, 23].

In summary, ITG typically affects older patients and does not have gender predilection. Hematologic malignancy (most commonly CLL) is present in over one-third of patients and a circulating paraprotein in close to two-thirds. Half of ITG patients are expected to recover kidney function with immunosuppressive therapy or chemotherapy. The proteomic profile of ITG is consistent with deposition of monotypic immunoglobulins and activation of the classical and terminal pathway of complement.

Conflict of interest statement. None declared.

References

A randomized controlled trial of oral heme iron polypeptide versus oral iron supplementation for the treatment of anaemia in peritoneal dialysis patients: HEMATOCRIT trial

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Abstract

Background. Preliminary clinical evidence suggests that heme iron polypeptide (HIP) might represent a promising, novel oral iron supplementation strategy in chronic kidney disease. The aim of this multi-centre randomized controlled trial was to determine the ability of HIP administration to augment iron stores in darbepoetin (DPO)-treated patients compared with conventional oral iron supplementation.

Methods. Adult peritoneal dialysis (PD) patients treated with DPO were randomized 1:1 to receive two capsules daily of either HIP or ferrous sulphate per os for 6 months. The primary outcome measure was transferrin saturation (TSAT). Secondary outcomes comprised serum ferritin, haemoglobin, DPO dose and responsiveness, and adverse events.

Results. Sixty-two patients were randomized to HIP (n = 32) or ferrous sulphate (n = 30). On intention-to-treat analysis, the median (inter-quartile range) TSAT was 22% (16–29) in the HIP group compared with 20% (17–26) in controls (P = 0.65). HIP treatment was not significantly associated with TSAT at 6 months on multivariable analysis (P = 0.95). Similar results were found on per-protocol analysis and subgroup analysis in iron-deficient patients. Serum ferritin levels at 6 months were significantly lower in the HIP group (P = 0.003), while the cost of HIP was 7-fold higher than that of ferrous sulphate. No other differences in secondary outcomes were observed.

Conclusions. HIP showed no clear safety or efficacy benefit in PD patients compared with conventional oral iron supplements. The reduction in serum ferritin levels and high costs associated with HIP therapy suggest that this agent is unlikely to have a significant role in iron supplementation in PD patients.

Keywords: anaemia; chronic kidney disease; heme iron polypeptide; iron; randomized controlled trial

Introduction

Treatment of anaemia with erythropoiesis-stimulating agents (ESAs) has resulted in major health benefits for individuals with end-stage kidney disease (ESKD), including improved quality of life, reduced blood transfusion requirements, decreased left ventricular mass, diminished sleep disturbance and enhanced exercise capacity [1–3]. However, ESA treatment results in a substantial increase in the iron demand for erythropoiesis [4, 5], such that as many as 90% of ESA-treated individuals require iron supplementation to sustain an optimal haematological response to ESAs [6, 7].

Numerous studies in haemodialysis populations have shown an advantage of intravenous over oral iron supplementation, as evidenced by enhanced body iron stores,