Noninfectious mixed cryoglobulinaemic glomerulonephritis associated with monoclonal gammopathy of undetermined significance

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Abstract

Background

Cryoglobulins are cold-precipitable immunoglobulins that may be found in association with systemic vasculitis including cryoglobulinaemic glomerulonephritis (CGN). Type 1 cryoglobulins consist of isolated monoclonal immunoglobulin (mIg), whereas mixed cryoglobulins are typically immune complexes comprising either monoclonal (type 2) or polyclonal (type 3) Ig with rheumatoid activity against polyclonal IgG. Only CGN related to type 1 cryoglobulins has been clearly associated with monoclonal gammopathy of undetermined significance (MGUS) using the conventional serum-, urine- or tissue-based methods of paraprotein detection.

Methods

We retrospectively assessed our patient cohort of CGN related to mixed (type 2 or 3) cryoglobulins for those with a monoclonal band on serum protein electrophoresis (SPEP), with exclusion of infectious cases and those involving malignant clonal plasma cell or B cell disorders.

Results

We identified four patients with a median age of 54 years, including three women. Two patients had type 2 cryoglobulinaemia, one had type 3 cryoglobulinaemia, and one lacked definitive typing of the serum cryoprecipitate. The serum monoclonal band identified on SPEP was IgM-κ in all four cases. Treatments included corticosteroids, cyclophosphamide, plasma exchange, and rituximab. At median 3.5 years’ follow-up, no patient had developed a haematological malignancy or advanced chronic kidney disease. Other potential causes of mixed cryoglobulinaemia were also present in our cohort, notably primary Sjögren’s syndrome in three cases.

Conclusions

Our study raises questions regarding the attribution of causation to MGUS, and the role of clonally directed therapies outside the setting of haematological malignancy.

Background

Cryoglobulinaemia is defined by the presence of circulating immunoglobulin (Ig) that aggregates in vitro at temperatures <37°C, and redissolves on rewarming (1). For cryoglobulinaemia to be
detected, blood sampling, clotting and serum separation by centrifugation must ideally be performed at 37°C, before serum storage at 4°C for up to seven days (2). Any significant cryoprecipitate (usually >0.05g/L or cryocrit >1%) may then be quantified and analyzed by electrophoresis and immunofixation after washing and redissolving at 37°C. The classification system for cryoglobulinaemia devised by Brouet and colleagues distinguishes three main types (3). Type 1 cryoglobulins consist of monoclonal Ig (mlg) or biclonal Ig, and occur in patients with clonal B cell or plasma cell disorders (4). So-called ‘mixed’ cryoglobulins are considered as immune complexes typically comprising either monoclonal (type 2) or polyclonal (type 3) Ig (mostly IgM) with rheumatoid factor activity against the Fc portion of polyclonal IgG. Infections are the commonest cause of mixed cryoglobulinaemia, notably hepatitis C virus (HCV) (5) and hepatitis B virus (HBV) (6), together with human immunodeficiency virus (HIV) and numerous other viral, bacterial, parasitic and fungal infections (1, 7). Noninfectious causes of mixed cryoglobulinaemia include autoimmune diseases, especially primary Sjögren’s syndrome (pSS) (8), and the malignant clonal disorders (9).

There is some uncertainty as to whether cryoglobulins are truly pathogenic in vivo, given that disease manifestations including systemic vasculitis occur in only a minority of patients with detectable cryoglobulinaemia (10). The systemic vasculitis is exemplified by cryoglobulinaemic glomerulonephritis (CGN), which can be classified as type 1 or mixed according to whichever type of cryoglobulin is found in association. Classic renal histological features of CGN, including membranoproliferative glomerulonephritis (MPGN), intracapillary ‘pseudothrombi’, crescents and small vessel vasculitis are nonspecific for cryoglobulinaemia as the underlying cause of glomerulonephritis (11). On the other hand, causation due to glomerular deposition of cryoglobulins may be strengthened by the appearance on electron microscopy (EM) of curvilinear microtubules, suggestive of aggregated cryoglobulins (12, 13), or immunohistochemistry showing light chain restriction of pseudothrombi in the case of (monotypic) type 1 CGN (14, 15).

Monoclonal gammopathy is diagnosed when mlg secreted into the circulation by a proliferating clone of plasma cells or B cells is detected by means of serum protein electrophoresis (SPEP), immunofixation (SIFE) or free light chain assays (SFLC), or urine protein electrophoresis (UPEP) or
immunofixation (UIFE) (16). Further evaluation is often required for the malignant clonal disorders, which include multiple myeloma, Waldenström’s macroglobulinaemia, B cell lymphoma and chronic lymphocytic leukemia. However, in most patients, monoclonal gammopathy of undetermined significance (MGUS) or another pre-malignant haematological disorder is diagnosed. Previous studies have established a clear association of type 1 CGN not only with malignant clonal disorders, but also with MGUS (14, 17-20). This has led to the inclusion of type 1 CGN within the disease classification of monoclonal gammopathy of renal significance (MGRS) (21-23). The term MGRS recognizes that certain renal lesions may be caused by nephrotoxic mlg produced by small (i.e. pre-malignant) plasma cell or B cell clones. This diagnostic concept is underscored in many cases by light chain restricted renal immunostaining (as noted above for type 1 CGN) (24). Yet surprisingly, type 2 CGN has also been included within MGRS (21-23), despite a weak association with MGUS in the published literature, and the inability to confirm light chain restriction owing to the polyclonal Ig component of type 2 cryoglobulins (11). We undertook this study to assess whether mixed (type 2 or 3) CGN can equally be associated with MGUS.

Methods
Cases were identified on retrospective review of clinical and pathological databases at our hospital for the period 2002-19, and were included if they met all the following criteria: (1) renal histology compatible with CGN; and both (2) cryoglobulinaemia >0.05 g/L and (3) a monoclonal band on SPEP at the time of diagnostic renal biopsy. Patients were excluded if they met any of the following criteria: (i) type 1 cryoglobulinaemia based on immunofixation of the cryoprecipitate and/or light chain restriction of renal biopsy tissue, as assessed by immunofluorescent staining of paraffin-embedded tissue after protease digestion (paraffin-IF) (25); (ii) a malignant clonal disorder based on tests comprising at a minimum bone marrow aspirate and trephine (BMAT) with flow cytometry of the aspirate and/or peripheral blood, and computed tomography with positron emission tomography (CT-PET); (iii) a potential infectious aetiology for mixed cryoglobulinaemia based on tests comprising at a minimum blood cultures, HBV surface antigen, and HBV core, HCV, and HIV antibodies; or (iv) vasculitis potentially due to causes other than cryoglobulinaemia based on serologies comprising at a
minimum anti-neutrophil cytoplasmic, anti-glomerular basement membrane, anti-double stranded DNA, anti-U₁-ribonucleoprotein, and anti-cardiolipin antibodies, and lupus anticoagulant.

Results

Of twenty patients with noninfectious mixed CGN in whom SPEP was performed, four had MGUS, comprising three females and one male (Table 1). Median age at presentation was 54 years (range 47 – 66 years). All patients had microscopic haematuria and proteinuria, with a median urinary protein creatinine ratio (uPCR) of 106mg/mmol (range 70-134mg/mmol). Renal function was significantly impaired in three of the four patients, with a median serum creatinine overall of 221μmol/L (range 80-292μmol/L) eGFR of 27mL/min per 1.73m² (range 15-70mL/min per 1.73m², MDRD). All patients had mildly reduced serum albumin levels, and serum complement C4 ± C3 levels were low in three patients. All three female patients had previously been diagnosed with pSS and showed seropositivity for antinuclear, SSA/Ro and SSB/La antibodies. In the weeks following diagnosis of CGN, Patient 2 underwent laparotomy for an incidental finding of cholangiocarcinoma (not previously reported in the setting of CGN (26)).

Histological features of CGN included MPGN in three patients, cellular crescents with arteriolar necrosis and thrombosis in one patient, and intracapillary pseudothrombi in three patients (Figure 1 and Table 2). Interstitial fibrosis ≥25% with mild glomerulosclerosis was also present in three cases. Immunohistochemistry showed variable IgG, IgM and C3 staining in capillary loops and the mesangium, with IgM and/or IgG staining of pseudothrombi in two cases. No case showed light chain restriction on paraffin-IF. EM was performed in three cases, revealing intracapillary curvilinear deposits in one case and unstructured deposits in the other two cases.

Serum biochemistry at presentation (Table 3) included a median cryoglobulin concentration of 0.43g/L (range 0.1-0.62g/L) in three cases, with a cryocrit of 9% in the fourth case. Immunofixation of the cryoprecipitate confirmed type 2 cryoglobulinaemia with a monoclonal IgM-k component in two patients and type 3 cryoglobulinaemia in one patient, and was not performed in the remaining patient. SPEP revealed generally small monoclonal bands of median concentration <1g/L (range <1 - 2g/L). In all four cases, the paraprotein was IgM-k, with an IgG-k paraprotein also present in one case.
(Patient 2). No patient showed bone marrow evidence of a malignant plasma cell or B cell disorder (Table 4).

Treatment consisted of corticosteroids in all patients, cyclophosphamide in three patients, plasma exchange in three patients, and rituximab in two patients, one of whom (Patient 4) later received maintenance therapy for a diagnosis of seronegative lupus nephritis with mycophenolate mofetil (Table 5). At a median follow-up of 3.5 years (range 1.5 – 11 years), no patient had developed a malignant clonal disorder or end-stage renal failure, or was deceased. Renal parameters were improved in all cases but one (Patient 4), with a median uPCR at last follow-up of 103mg/mmol (range 24 – 302mg/mmol), serum creatinine of 133μmol/L (range 62 - 168μmol/L), and eGFR of 45mL/min per 1.73m² (range 39-90mL/min per 1.73m²). Serum C4 ± C3 levels remained low in the three patients with this finding on presentation. Cryoglobulinaemia was undetectable at last follow up in all four patients, and SPEP/UPEP was negative. However, SIFE revealed an IgM-k paraprotein (<1g/L) in one patient, and the SFLC ratio was elevated at 2.09 in one other patient, with no evidence for persisting monoclonal gammopathy in the remaining two patients.

Discussion

We report a series of four patients with noninfectious mixed CGN in whom MGUS was diagnosed using the conventional methods for paraprotein detection (16, 27). One in every five patients assessed in our hospital cohort of noninfectious mixed CGN was found to have MGUS, although the true incidence of this association remains uncertain owing to a paucity of data in the major published series (11, 28, 29). This is partly because of limited biochemical analysis in these studies, which tended to focus only on typing of the cryoglobulin by means of immunofixation of the cryoprecipitate. Whilst this remains a highly sensitive technique for detecting mlg (>0.05g/L) in patients with type 1 or 2 cryoglobulinaemia, for example in comparison to SPEP (>0.5g/L) (30), its role in diagnosis of MGUS is not established. Thus all 20 patients in one series of noninfectious mixed CGN were shown to have type 2 CGN, with monoclonal gammopathy reported in 18 patients, yet without reference to cryoglobulin quantitation, SPEP, SIFE, SFLC, UPEP or UIFE (29). This data was also not available in a recent series of 80 patients with noninfectious mixed CGN comprising 75 patients with type 2 CGN.
Conditions other than MGUS were present in our cohort potentially accounting for the development of mixed CGN. Thus pSS, which represents the commonest cause of mixed cryoglobulinaemia/CGN after HCV infection (8, 9, 11, 28, 29), was present at time of diagnostic renal biopsy in three patients (conforming to current EULAR/ACR criteria (31)). pSS involves a disease process of continuous polyclonal B cell activation, with malignant transformation of B cell clones in some cases, based on the increased incidence of lymphoma in patients with pSS (32, 33) (especially those with mixed cryoglobulinaemia (34, 35)). Of note, MGUS may also be more common in patients with pSS, and may confer an increased risk of developing lymphoma (36, 37) or myeloma (38). Yet co-presence of pSS and MGUS has not been previously reported in non-infectious mixed CGN. These observations raise questions regarding the underlying pathophysiological processes responsible for type 2 CGN in our three patients with pSS. The patient in our cohort who did not have pSS was one of three cases in which only a very small monoclonal band (<1g/L) was identified, emphasizing the need for further studies in which robust biochemical analysis is undertaken, if the association of mixed CGN with MGUS is to become established.

It remains unclear from our study whether the identification of MGUS in a patient with noninfectious mixed CGN provides any guide to treatment and prognosis. A designation of MGRS would imply the need for clonally targeted therapies to be considered in preference to conventional immunosuppression, with the primary aim of improving renal outcomes (24). Evidence for this approach in patients with noninfectious mixed CGN consists largely of retrospective studies of rituximab, mostly involving patients with type 2 CGN and a monoclonal IgM-k cryoglobulin (11, 28, 29, 39). As in previous series (11, 28), our cohort received multiple therapies including rituximab but also conventional immunosuppressive agents (11, 28). Outcomes were generally favourable, and of the two patients with chronic kidney disease stage 3B at last follow-up, both had shown significant (25-40%) interstitial fibrosis on pre-treatment biopsies (potentially due to pSS-associated interstitial nephritis in one of these cases). Given previous, larger cohorts of noninfectious mixed CGN showing ESKD rates of 9-10% at 4 years (11, 29), our study
possibly indicates that MGUS does not always confer a treatment-resistant course. No patient in our series received bortezomib, for which a single instance of use in refractory noninfectious mixed CGN is reported, in a patient with type 2 CGN and a monoclonal IgM-k component, but no detectable monoclonal band on SPEP (40).

Conclusions
Our study is the first to show conclusively that MGUS may be present in a subset of patients with noninfectious mixed CGN. Even where this is the case, a designation as MGRS may be open to question, given the presence in our cohort of other potential aetiologies for mixed CGN, including pSS in three out of four patients.

Declarations
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Authors contributions: A Flavell and T Barbour collected & analysed the data and prepared the manuscript. R Fullinfaw assisted with data collection & interpretation. M Finlay collected pathology data and images. E Smith and S Holt reviewed the manuscript.

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Tables
Table 1. Demographic and renal clinical features at time of renal biopsy

| Patient | Age (Years) | Sex | Extrarenal clinical features | Comorbid conditions | Urine studies | Red cells | PCR (<30mg/mmol) | ACR (<3.5mg/mmol) |
|---------|-------------|-----|-------------------------------|---------------------|---------------|-----------|-----------------|-------------------|
| 1       | 47          | F   | Purpura, arthritis           | pSS                 | Pos           | 70        | 33              |
| 2       | 60          | F   | Purpura, benign lymphadenopathy | pSS, CC             | Pos           | 126       | 75              |
| 3       | 66          | M   | -                             | -                   | Pos           | 86        | 55              |
| 4       | 47          | F   | Purpura, arthritis           | pSS, hypoGG         | Pos           | 134       |                 |

PCR - protein creatinine ratio; ACR - albumin creatinine ratio; eGFR - estimated glomerular filtration rate; ANA - antinuclear antibodies; pSS - primary Sjögren's syndrome; CC - cholangiocarcinoma; hypoGG - hypogammaglobulinaemia
* Modified diet in renal disease (MDRD)
^Nephrotic syndrome

Table 2. Renal histology

| Patient | N° glomeruli (N° globally sclerosed) | Pattern of glomerular inflammation | Arteriolar necrosis, thrombosis | Interstitial inflammatory infiltrate | Interstitial fibrosis (<5%) | Pseudothromb |
|---------|-------------------------------------|-----------------------------------|---------------------------------|-----------------------------------|---------------------------|--------------|
| 1       | 25 (0)                              | Early MPGN                        | No                              | Patchy, mild                     | <5%                       | Yes          |
| 2       | 18 (6)                              | Crescents (5 cellular, 1 fibrocellular, 2 fibrous) | Yes | Light, chronic | 40% | No |
| 3       | 20 (5)                              | MPGN, endocapillary (CD68+) | No | Light, chronic | 25% | Yes |
| 4       | 18 (2)                              | MPGN                              | No                              | Moderate                          | 25%                       | Sparse       |

MPGN - membranoproliferative glomerulonephritis; IHC - immunohistochemistry; IF - immunofluorescence; IF - electron microscopy; κ - kappa; λ - lambda
### Table 3. Biochemistry at time of renal biopsy

| Patient | Serum cryoglobulin |  |  |  |  |
|---------|-------------------|---|---|---|---|
|         | Concentration     | Type | RF  | SPEP | SIFE |
|         | (g/L)             | (g/L) | (g/L) |     |     |
| 1       | 0.1               | 2 or 3 | Neg |     |     |
| 2       | 0.43              | 2     | Pos | <1  | IgM-κ, IgG-κ |
| 3       | 0.62              | 3     | Pos | <1  | IgM-κ |
| 4       | 9% cryocrit       | 2     | Pos | <1  | IgM-κ |

RF - rheumatoid factor; SPEP - serum protein electrophoresis; SIFE - serum immunofixation; SFLC - serum free light chains; UPEP/UIFE - urine protein electrophoresis/immunofixation; mIg - monoclonal immunoglobulin; pIg - polyclonal immunoglobulin

*Freelite assay, The Binding Site Group, Birmingham, UK*

### Table 4. Bone marrow aspirate and trephine

| Patient | Trephine | Aspirate |  |
|---------|----------|----------|---|
|         | Cellularity | Immunohistochemistry | Lymphoid cells (5-20%) |
|         | Plasma cells (<5%) | Lymphoid aggregates |  |
| 1       | Normal   | <5%      | One small  | 16% |
| 2       | Normal   | <5%      | Two small  | 9%  |
| 3       | Mildly hypercellular | <5% | None | 9%  |
| 4       | Normal   | <5%      | None      | 5%  |

Figures
Table 5. Treatment and last follow-up

| Patient | Treatment received | Recurrent vasculitis | Urine PCR (<30mg/mmol) | Serum creatinine (μmol/L) | eGFR* (mL/min per 1.73 m²) | Serum cryoglobulin |
|---------|--------------------|----------------------|------------------------|--------------------------|----------------------------|-------------------|
| 1       | CS/PE/CYC          | No                   | 41                     | 62                       | 90                         | Ne                |
| 2       | CS/PE/CYC/AZA      | No                   | 24                     | 159                      | 30                         | Ne                |
| 3       | CS/PE/RTX          | Yes                  | 165                    | 168                      | 38                         | Ne                |
| 4       | CS/CYC/RTX/MS      | No                   | 302                    | 106                      | 51                         | Ne                |

PCR - protein creatinine ratio; eGFR - estimated glomerular filtration rate; SPEP/SIFE - serum protein electrophoresis/immunofixation; κ - kappa; λ - lambda; CS - corticosteroids; PE - plasma exchange; CYC - cyclophosphamide; AZA - azathioprine; RTX - rituximab; MS - mycophenolate sodium

* MDRD

† Freelite, UK
Figure 1

Histology. Light microscopy in patient 1 with a) periodic acid-Schiff stain and b) silver stain showing MPGN with double contours and striking intraluminal, PAS-positive pseudothrombi. Equal (+++) intensity of paraffin-IF staining of pseudothrombi for c) 1 and d) 2 light chain.

In patient 2, e) silver stain showing a small cellular crescent with necrosis, and f) haematoxylin and eosin stain of a small artery with concentric intimal arteritis.

Magnification x40.