Renal Involvement in Essential Mixed Cryoglobulinemia

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Franco Ferrario

Up to the end of the 1980s, the cause of about 30% of both type II and III mixed cryoglobulinemias (MC) in patients was not known, and this subgroup of patients were referred to as having essential mixed cryoglobulinemia. Essential mixed cryoglobulinemia was characterized clinically by systemic signs, mainly purpura, arthralgias, and fever, together with hepatic, neurologic, and renal symptoms. During this decade, antibodies against hepatitis C virus (HCV) antigens and HCV RNA (which is a marker of active viremia) have been detected in the serum of up to 90% of these patients.

Only when a monoclonal rheumatoid factor, usually an immunoglobulin M k (IgMk), is the anti-IgG component of the mixed cryoglobulinemia (type II MC) does this distinctive glomerular and vascular involvement of the kidney occur. The most frequent histologic picture, especially in the acute stages, is a membranoproliferative glomerulonephritis (MGN) with subendothelial deposits, with some characterizing features both by light and electron microscopy. However, a less distinctive picture of lobular MGN is found at biopsy in 20% of patients, and of a mesangiproliferative glomerulonephritis in another 20%. In all cases, the two components of MC, IgG, and IgM, together with complement, are found by immunofluoroscopy.

The clinical picture varies during the long-term course of the disease, being characterized by periods of temporary reactivation (nephritic or nephrotic syndrome, sometimes with rapidly occurring renal insufficiency) and long-lasting periods of partial remission. Only infrequently does end-stage renal failure develop; however, mortality as a result of the other complications of the systemic disease (mainly cardiovascular) is rather frequent.
During acute flare-ups, antiviral treatment (interferon-\(\alpha\)) is insufficient to control the renal disease, even when it reduces viremia. Steroids, usually associated with immunosuppressive drugs (cyclophosphamide), are then necessary to control renal disease. Hepatitis C virus can infect B lymphocytes and stimulate them to synthesize the cryoprecipitating polyclonal rheumatoid factors responsible for type III M C. In some patients with this polyclonal B-cell activation, additional but as yet uncharacterized events might induce the shift to abnormal proliferation of a clone of B cells, producing a monoclonal IgM rheumatoid factor. Thus, a type II M C is induced that can be considered a lymphoproliferative disorder. It has been suggested that the IgM produced by this permanent clone of B cells has affinity for the glomerular matrix and can deposit, in the glomerulus together with the IgG to which it binds in the blood, IgG that probably acts as an anti-HCV antigen antibody.

### CLASSIFICATION OF CRYOGLOBULINEMIAS AND ASSOCIATED DISEASES

<table>
<thead>
<tr>
<th>Type I: single monoclonal IgA, IgG, or IgM</th>
<th>Type II: polyclonal IgG bound to monoclonal anti-IgG rheumatoid factor*</th>
<th>Type III: polyclonal IgG bound to polyclonal anti-IgG rheumatoid factor*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiple myeloma</td>
<td>B-lymphocytic neoplasm</td>
<td>Autoimmune diseases: SLE, polyarteritis nodosa, rheumatoid arthritis, scleroderma, (\varphi)igren's syndrome, and Henoch-Schönlein purpura</td>
</tr>
<tr>
<td>Waldenström's macroglobulinemia</td>
<td>Diffuse lymphoma</td>
<td>Infections diseases: mononucleosis, cytomegalovirus, hepatitis B, subacute bacterial endocarditis, leprosy, malaria, schistosomiasis, toxoplasmosis, AIDS</td>
</tr>
<tr>
<td>Chronic lymphocytic leukemia</td>
<td>Chronic lymphocytic leukemia</td>
<td>Miscellaneous diseases: primary proliferative glomerulonephritis, lymphoma, chronic hepatitis, biliary cirrhosis</td>
</tr>
<tr>
<td>Idiopathic monoclonal gammopathy</td>
<td>SJögren's syndrome</td>
<td>Essential</td>
</tr>
</tbody>
</table>

* Usually IgM.

From Brouet and coworkers [1]; with permission.

### FIGURE 9-1
Classification of cryoglobulinemias and associated diseases as proposed by Brouet and coworkers in 1974 [1]. Up to the end of the 1980s, the cause of about 30% of both types II and III mixed cryoglobulins was not clear, and this group of mixed cryoglobulinemias was called essential [2,3]. As indicated in Figure 9-4, it now is evident that most essential mixed cryoglobulinemias are associated with hepatitis C virus infection.

### FIGURE 9-2
Correct methodology for detecting circulating cryoglobulins. Cryoglobulins are immunoglobulins that precipitate reversibly from cooled serum.

### DETECTION OF CIRCULATING CRYOGLOBULINS AND DETERMINATION OF CRYOPRECIPITATE

- Prewarm syringe, needle, and tubes at 37°C.
- Take 20 mL of whole blood and put it immediately at 37°C.
- Incubate for 2 h at 37°C to allow clotting.
- Centrifuge twice at 1700 g X 10 at 37°C to discard platelets and erythrocytes.
- Cryoglobulins precipitate reversibly from cooled serum.
- Keep serum at 4°C in a conical graduate tube.
- Look at the serum after 7 d.
- Centrifuge serum at 400 g X 10 at 4°C and calculate the cryocrit as the percentage of packed cryoglobulins and serum ratio.
Immunoglobulin composition and clonality of mixed cryoglobulins characterized by immunofixation. The cryoglobulins (isolated, as indicated in Fig. 9-2) are resuspended in three volumes of cold phosphate-buffered saline at 4°C and then washed by centrifuging at 1700 g for 10 minutes at 4°C; the supernatant is discarded. This procedure is repeated at least four times. Next, the cryoprecipitate is solubilized in three volumes of phosphate-buffered saline at 37°C before gel electrophoresis is performed.

A, Example of type II mixed cryoglobulin; the immunoglobulin M rheumatoid factor contains $\kappa$ but not $\lambda$ light chains and therefore is monoclonal. B, Example of type III mixed cryoglobulin; the immunoglobulin M rheumatoid factor contains both $\kappa$ and $\lambda$ light chains and therefore is polyclonal. (Beckman Paragon® IFE gel.)

### PREVALENCE OF HEPATITIS C VIRUS AND HEPATITIS C VIRUS RNA IN ESSENTIAL AND SECONDARY MIXED CRYOGLOBULINEMIAS*

<table>
<thead>
<tr>
<th>Study</th>
<th>Types of mixed cryoglobulinema</th>
<th>Serum HCV antibodies</th>
<th>Serum HCV RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Patients tested, n</td>
<td>Positive patients, %</td>
</tr>
<tr>
<td>Ferri et al. [5]</td>
<td>II and III EMC</td>
<td>52</td>
<td>54</td>
</tr>
<tr>
<td>Galli et al. [6]</td>
<td>II and III EMC</td>
<td>129</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>63</td>
<td>70</td>
</tr>
<tr>
<td>Pechère-Bertschi et al. [7]</td>
<td>II and III EMC</td>
<td>15</td>
<td>87</td>
</tr>
<tr>
<td>Agnello et al. [8]</td>
<td>II EMC</td>
<td>19</td>
<td>42</td>
</tr>
<tr>
<td>Misiani et al. [9]</td>
<td>II EMC</td>
<td>75</td>
<td>96</td>
</tr>
<tr>
<td>Pasquarriello et al. [10]</td>
<td>II EMC with GN</td>
<td>26</td>
<td>100</td>
</tr>
<tr>
<td>Cacoub et al. [11]</td>
<td>II and III EMC</td>
<td>63</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>SM C</td>
<td>52</td>
<td>27</td>
</tr>
<tr>
<td>Bichard et al. [12]</td>
<td>II and III EMC</td>
<td>15</td>
<td>93</td>
</tr>
<tr>
<td>D’Amico, Unpublished data</td>
<td>II EMC</td>
<td>41</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td>II EMC with GN</td>
<td>28</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td>III EMC</td>
<td>13</td>
<td>77</td>
</tr>
</tbody>
</table>

*According to published data [4].

EMC—essential mixed cryoglobulinemia; GN—glomerulonephritis; HCV—hepatitis C virus; SMC—secondary mixed cryoglobulinemia.

### FIGURE 9-4

Second-generation enzyme-linked immunosorbent assay has been used by all the authors listed here (with the exception of Agnello and coworkers [9], who used a recombinant immunoblot assay) to measure anti-hepatitis C virus (HCV) antibodies. The prevalence of positivity of HCV RNA in the 15 patients studied by Bichard and coworkers [12] increased from 60% to 93% when cryoprecipitate from serum was tested.
FREQUENT EXTRARENAL SIGNS IN PATIENTS WITH TYPES II AND III MIXED CRYOGLOBULINEMIA

<table>
<thead>
<tr>
<th>Signs and symptoms</th>
<th>Prevalence during course of disease, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cutaneous purpura</td>
<td>= 95</td>
</tr>
<tr>
<td>Arthralgias</td>
<td>= 85</td>
</tr>
<tr>
<td>Fever</td>
<td>= 60</td>
</tr>
<tr>
<td>Hepatosplenomegaly</td>
<td>= 95</td>
</tr>
<tr>
<td>Neuropathy</td>
<td>= 40</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>= 30</td>
</tr>
</tbody>
</table>

FIGURE 9-5
Extrarenal signs frequently present in patients with types II and III mixed cryoglobulinemia, either essential or due to hepatitis C virus infection, with or without cryoglobulinemic nephropathy. In patients with cryoglobulinemic nephropathy, the systemic signs usually appear months or years before renal complications develop. The onset of these signs, however, may be concomitant with or even subsequent to the onset of renal signs. Abdominal pain is due to mesenteric vasculitis [13].

FIGURE 9-6
A purpuric rash of the legs in a patient with mixed cryoglobulinemia associated with hepatitis C virus infection.

DISTINCTIVE FEATURES OF MEMBRANOPROLIFERATIVE GLOMERULONEPHRITIS, OR CRYOGLOBULINEMIC GLOMERULONEPHRITIS

Exudative component
The major constituent of intracapillary proliferation is an infiltration of leukocytes, mainly monocytes, that sometimes is massive.

Intraluminal thrombi
Huge deposits of cryoglobulins called intraluminal thrombi sometimes fill the capillary lumen.

Interposition of monocytes in the double contour of the capillary wall
Monocytes, in close contact with the subendothelial deposits of cryoglobulins, are interposed between the glomerular basement membrane and the newly formed membranelike material, to give the double-contoured appearance of the capillary wall, whereas peripheral interposition of mesangial matrix and cells is moderate.

Structured crystallloid deposits on electron microscopy
Intraluminal and subendothelial deposits of cryoglobulins sometimes show a specific fibrillar structure on electron microscopy.

Vasculitis of small and medium-sized arteries
Necrotizing arteritis, without concomitant features of segmental necrotizing glomerulonephritis, is found in one third of patients.

FIGURE 9-7
The distinctive features of the membranoproliferative glomerulonephritis. This disorder, called cryoglobulinemic glomerulonephritis, occurs only in patients with type II mixed cryoglobulinemia, especially in the acute stage of the disease [4,14]. In about 20% of patients with type II mixed cryoglobulinemia, a less distinctive picture of lobular membranoproliferation is found, whereas an additional 20% exhibit mild mesangial proliferation. These various types of histologic lesions can be found by repeat biopsies in the same patient during different stages of the disease.
Membranoproliferative exudative glomerulonephritis in patients with type II mixed cryoglobulinemia. The marked endocapillary hypercellularity also is due to massive intraglomerular infiltration of mononuclear leukocytes, mainly monocytes (Fig. 9-9). Mesangial cell proliferation and mesangial matrix expansion are mild. Many loops show a thickened glomerular capillary wall, with frequent double-contoured basement membrane. (Trichrome stain × 250.)

Immunohistochemical staining with anti-monocyte-macrophage antibody (CD68). This reaction confirms that the intracapillary hypercellularity is due mainly to accumulation of these mononuclear leukocytes. Their average number in acute stages of cryoglobulinemic glomerulonephritis is four times greater than in severe proliferative lupus nephritis [15]. (Immunoperoxidase × 250.)

Monocyte in close contact with a massive endocapillary deposit showing phagocytic activity. (Uranyl acetate-lead citrate × 8000.) (Courtesy of Department of Pathology, San Carlo Borromeo Hospital, Milan, Italy.)

Presence of huge intracapillary deposits typical of cryoglobulinemic glomerulonephritis. These huge intracapillary deposits are called intraluminal thrombi. The only possible differential diagnosis is with glomerulonephritis secondary to Waldenström macroglobulinemia. The glomerulus shows morphologic lesions similar to those seen in Figure 9-8, characterized by marked endocapillary hypercellularity mainly a result of mononuclear leukocyte accumulation. Two large intraluminal deposits, stained in green and red, are evident in the part of the glomerular tuft opposite the vascular pole. It is now well known that these deposits are expressions of acute and massive intracapillary precipitation of circulating cryoglobulins. (Trichrome stain × 250.)
Electron microscopy of subendothelial and endocapillary deposits showing an amorphous structure. In a minority of cases, as illustrated here, a specific annular and cylindrical structure is shown. This structure is identical to that seen in the in vitro precipitate of the same patients and consists of cylinders 100- to 1000-µm long, with a hollow axis, appearing in cross-sections as annular bodies [16]. (Uranyl acetate-lead citrate × 22,000.) (Courtesy of Department of Pathology, San Carlo Borromeo Hospital, Milan, Italy.)

Silver stain showing the double-contoured appearance of the basement membrane. This morphologic aspect is diffuse and more clearly visible than in idiopathic membranoproliferative glomerulonephritis or lupus nephritis. (Silver stain × 250.)

Interposition of monocytes in cryoglobulinemic glomerulonephritis. Two monocytes containing lysosomes are interposed, together with electron-dense subendothelial deposits, between the glomerular basement membrane and the newly formed basement-membrane-like material of the double-contoured capillary wall. The interposition of monocytes is a distinctive feature of cryoglobulinemic glomerulonephritis [17,18]. Mesangial matrix and mesangial cell interposition, however, usually are less evident than in idiopathic membranoproliferative glomerulonephritis, as is glomerular sclerosis. (Uranyl acetate-lead citrate × 8000.) (Courtesy of Department of Pathology, San Carlo Borromeo Hospital, Milan, Italy.)

Morphologic pattern of lobular glomerulonephritis. This pattern is present in 20% of cases, characterized by intense mesangial proliferation and peripheral mesangial matrix expansion associated with centrolobular sclerosis. This histologic picture is indistinguishable from that of idiopathic membranoproliferative glomerulonephritis type I, except for the presence of some degree of monocyte infiltration. (Trichrome × 250.)
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**FIGURE 9-16**
The glomerulus showing only mild mesangial proliferation and mesangial matrix expansion. Thickening of the glomerular basement membrane is not evident. This picture frequently is present in cases clinically characterized only by mild urinary abnormalities (inactive phase). Moreover, in many cases in which a biopsy is taken during the acute phase of the disease with typical membranoproliferative patterns with or without thrombi, a second renal biopsy will show clear regression of the morphologically acute lesions with only mild mesangiproliferative alteration. (Trichrome × 250.)

**FIGURE 9-17** (see Color Plate)
The pattern of immunohistologic glomerular staining varies according to the different glomerular patterns seen on light microscopy. **A**, Diffuse granular subendothelial deposits along the capillary walls, with or without very rare intraluminal thrombi. (Immunoglobulin M × 250). **B**, Intense massive staining of the deposits totally filling the capillary lumina. Faint and irregular parietal deposits also are present. (Immunoglobulin × 250.) **C**, Parietal deposits with more evident peripheral lobular distribution. (Immunoglobulin × 250.) The components of mixed cryoglobulinemia immunoglobulin M and G, usually associated with C3, are the most frequently found immunoreactants.
Systemic Diseases and the Kidney

FIGURE 9-18
Interstitial infiltrates having different degrees of intensity and diffusion. When present, these infiltrates are composed not only of T lymphocytes and monocyte macrophages, as in most glomerular diseases, but also of B lymphocytes. (Periodic acid–Schiff reaction × 100.)

FIGURE 9-19 (see Color Plate)
Arteritis of small and medium-sized arteries. In about one third of cases an arteritis of small and medium-size arteries also is present. The artery shows diffuse fibrinoid necrosis of the vessel wall (in red) and intraparietal and perivascular leukocyte infiltration. It is worth emphasizing that even in the presence of renal arteritis we have never found in patients with cryoglobulinemia a picture of necrotizing crescentic glomerulonephritis, now considered a specific aspect of capillaritis in primary vasculitis (antineutrophil cytoplasm antibody–associated). This finding suggests that the vasculitic damage is limited to arterial vessels of larger size. (Trichrome × 100.)

### Renal Syndrome at Presentation in Patients with Cryoglobulinemic Glomerulonephritis and Associated Histologic Lesion

<table>
<thead>
<tr>
<th>Renal Syndrome</th>
<th>Patients, %</th>
<th>Frequent histologic features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolated proteinuria with microscopic hematuria, sometimes associated with moderate chronic renal insufficiency</td>
<td>≈55</td>
<td>Membranoproliferative glomerulonephritis (MPGN), with moderate infiltration of monocytes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lobular MPGN</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mesangiproliferative glomerulonephritis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MPGN with leukocytic infiltration, or intraluminal thrombi owing to abrupt massive precipitation of cryoglobulins, usually associated with renal and systemic vasculitis, or both</td>
</tr>
<tr>
<td>Acute nephritic syndrome, sometimes complicated by acute oliguric renal failure</td>
<td>≈25</td>
<td>MPGN, frequently of lobular type, with some infiltration of monocytes</td>
</tr>
<tr>
<td>Nephrotic syndrome</td>
<td>≈20</td>
<td></td>
</tr>
</tbody>
</table>

Renal syndrome at presentation in patients with cryoglobulinemic glomerulonephritis and associated histologic lesion. During the course of this disease, both the systemic and renal signs may vary remarkably, with periods of exacerbation alternating with periods of quiescence. Very often, exacerbation of the extrarenal signs is associated with exacerbation of renal disease (recurrent episodes of nephritic or nephrotic syndrome); however, a flare-up of renal disease may occur even in the absence of exacerbation of the extrarenal signs. Partial or total prolonged remission occurs spontaneously or after treatment in 10% to 15% of patients. Arterial hypertension frequently is severe and is present in most patients with cryoglobulinemic nephropathy.
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LABORATORY ABNORMALITIES IN ESSENTIAL MIXED CRYOGLOBULINEMIA

Circulating cryoglobulins
Cryocrits ranging from 2% to 70%, with large variations during the course of the disease
Hypocomplementemia
Very low levels of early C components (C1q and C4) and CH50; slightly low levels of C3; and high levels of late C components, C5 and C9

CLINICAL OUTCOMES OF 105 PATIENTS STUDIED IN THREE MILAN HOSPITALS FROM 1966 TO 1990

49% cumulative 10-year probability of survival, without renal failure
40% of patients died, mostly from cardiovascular diseases, liver failure, or infections
14% of patients progressed to chronic renal failure and required dialysis
14% of patients achieved complete and prolonged remission of renal symptoms

FIGURE 9-21
Relevant laboratory abnormalities in “essential” mixed cryoglobulinemia. During the course of this disease, cryoglobulins may temporarily become undetectable. Low levels of serum C4 cannot be corrected by treatment. Low levels of C3 frequently are found during clinical flare-ups and can be corrected by treatment.

TREATMENT OF ACUTE RENAL EXACERBATIONS OF CRYOGLOBULINEMIC GLOMERULONEPHRITIS AND VASCULITIS

Steroids are used to control inflammatory renal and systemic involvement
Cytotoxic drugs are used to block production of new cryoglobulins by the specific lymphocytic clone that produces the monoclonal immunoglobulin M k RF, and therefore, the precipitating cryoglobulins
Plasma exchange is used to remove circulating cryoglobulins from the blood before they deposit in the glomerulus and arterial walls

PROPOSED TREATMENT FOR MIXED CRYOGLOBULINEMIA ASSOCIATED WITH HEPATITIS C VIRUS INFECTION

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dosage</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interferon-α</td>
<td>3.0–6.0 MU, 3 times weekly</td>
<td>6–12 mo</td>
</tr>
<tr>
<td>Steroids</td>
<td>Methylprednisolone, 0.75–10 mg/d, intravenously</td>
<td>3 d</td>
</tr>
<tr>
<td></td>
<td>Prednisone, 0.5 mg/kg of body weight tapered over a few weeks until maintenance dose of 10–15 mg/d is achieved</td>
<td>6 mo</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>2 mg/kg of body weight</td>
<td>3–4 mo</td>
</tr>
<tr>
<td>Plasma exchange</td>
<td>Exchanges of 3 L of plasma, 3 times weekly</td>
<td>2–3 wk</td>
</tr>
</tbody>
</table>

FIGURE 9-22
The clinical outcomes in 105 patients studied in three hospitals in Milan, Italy, between 1966 and 1990. The median total follow-up time from clinical onset was approximately 11 years [19].

FIGURE 9-23
This approach to treatment of the acute renal exacerbations of cryoglobulinemia and vasculitis used previously when the viral cause of the disease was unknown is still valid now that the viral cause is evident. It is a common experience that the antiviral agent interferon-α, when given alone, does not control renal complications in the acute stage of the disease [20].

FIGURE 9-24
The proposed treatment for mixed cryoglobulinemia associated with hepatitis C virus infection in the presence of severe acute signs of renal involvement, i.e., glomerulonephritis and vasculitis. Plasma exchange is used only when acute renal insufficiency caused by massive precipitation of cryoglobulins is present. Interferon-α is given for more than 6 months only when negation of hepatitis C virus RNA is achieved in the first months, suggesting a beneficial effect on the viremia. Only the antiviral treatment with interferon-α eventually associated with low doses of steroids to control the systemic signs of mixed cryoglobulinemias should be given if renal involvement is mild. The association of interferon-α with another antiviral agent ribavirin, 0.6 to 1.0 g/d orally, now is being tested in patients with hepatitis C virus infection, with promising results [20].
We thank Dr. M. P. Rastaldi of the Division of Nephrology and Drs. E. Schiaffino and R. Boeri of the Department of Pathology of the Hospital of San Carlo Borromeo for their help.

References