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In Practice

Hepatitis C Virus Infection, Mixed Cryoglobulinemia, and Kidney Disease

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Hepatitis C virus (HCV) may instigate mixed cryoglobulinemia; the most significant accompanying kidney lesion is type I membranoproliferative glomerulonephritis, usually occurring in the context of type II mixed cryoglobulinemia. Additionally, recent data support a link between HCV infection and proteinuria in population-based studies, raising the possibility that kidney diseases associated with HCV may be more common than previously thought. A number of strategies have been used to treat HCV-related glomerulonephritis, including antiviral agents, immunosuppressive therapies such as corticosteroids and cytotoxic agents, and plasma exchange. Limited but encouraging data about the utility of antiviral treatment in the setting of HCV-associated glomerulonephritis exist, with one pooled analysis noting a sustained viral response of 42%, albeit with significant heterogeneity. Immunosuppressive therapy may be most useful for cryoglobulinemic kidney disease, with individualized approaches considered for the treatment of HCV-associated cryoglobulinemic glomerulonephritis based on the level of proteinuria and kidney failure. Of note, rituximab, a chimeric monoclonal antibody that blocks CD20 receptors on B cells, has been reported to be effective for the treatment of mixed cryoglobulinemia symptoms, including glomerulonephritis.
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INDEX WORDS: Hepatitis C virus; cryoglobulinemia; proteinuria; glomerulonephritis; kidney disease; interferon.

CASE PRESENTATION

A 74-year-old man was admitted with hypertension and bilateral lower-extremity edema. His medical history included palpable purpura of the legs, chronic hepatitis C virus (HCV) infection, and chronic kidney disease stage 3 (serum creatinine, 1.3 mg/dL, consistent with estimated glomerular filtration rate [eGFR] of 54 mL/min/1.73 m^2, using the 4-variable MDRD [Modification of Diet in Renal Disease] Study equation). At admission, laboratory results included urinary protein excretion of 5 g/d, hypoalbuminemia (albumin, 2.5 g/dL), serum creatinine level of 1.97 mg/dL (eGFR, 33 mL/min/1.73 m^2), and active urinary sediment (microscopic hematuria and red blood cell casts). Serum C4 levels were low (7 mg/dL; reference range, 20-50 mg/dL), cryocrit was 20%, and cryoglobulin characterization showed type II (immunoglobulin G [IgG]–IgM-κ [ie, with κ light chain]) mixed cryoglobulinemia. The patient tested negative for hepatitis B virus markers and positive for anti-HCV antibody (although HCV RNA was undetectable). A percutaneous kidney biopsy showed features of membranoproliferative glomerulonephritis (MPGN; 5 of 10 glomeruli), with numerous subendothelial deposits and intracapillary thrombi; global hyalinosis was found in 5 glomeruli. We initiated treatment with intravenous methylprednisolone, 500 mg, once daily for 3 days, followed by oral prednisone, 40 mg daily, and also gave oral cyclophosphamide (50 mg daily). We observed an initial improvement, with serum creatinine level decreasing to 1.5 mg/dL (eGFR, 46 mL/min/1.73 m^2) 1 month after hospital discharge. However, the patient did not tolerate the immunosuppressive regimen, developing acute bronchitis 2 months after hospital discharge and resulting in a course of amoxicillin and discontinuation of cyclophosphamide therapy. The following month, he developed a urinary tract infection with *Klebsiella oxytoca* and prednisone dosage was tapered to 10 mg daily. At month 4, he was readmitted with acute pulmonary edema and serum creatinine level of 4.96 mg/dL (eGFR, 12 mL/min/1.73 m^2). Active urinary sediment and nephrotic proteinuria persisted. He initiated hemodialysis therapy acutely and currently is doing well on maintenance hemodialysis therapy.

INTRODUCTION

Cryoglobulinemia is a pathologic condition in which the blood contains immunoglobulins that have the property of reversible precipitation from human serum cooled to 4°C. The discovery in human serum of proteins with this property was made by Wintrobe and Buell.[1] In 1947, Lerner and Watson[2] found that these proteins were gamma globulins and introduced the term cryoglobulins (cold precipitable serum globulin). A definite nosographic placement of cryoglobulinemic disease within the vast family of systemic vasculitis was made by Meltzer et al,[3] who first described the clinical syndrome of essential mixed cryoglobulinemia, characterized by purpura, weak-

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Table 1. Classification and Clinical Features of Cryoglobulinemias

Type	Composition	Clinical Associations
Type I cryoglobulinemia	Monoclonal Ig, mainly IgG, IgM, or IgA (self-aggregated)	Lymphoproliferative disorders (mainly Waldenström macroglobulinemia)
Type II mixed cryoglobulinemia	Monoclonal IgM (or IgG or IgA) having RF activity and polyclonal Ig (mainly IgG)	Infections (mainly HCV), autoimmune disorders, lymphoproliferative disorders, or essential (idiopathic)
Type III mixed cryoglobulinemia	Polyclonal IgM (or IgG or IgA) having RF activity and polyclonal Ig (mainly IgG)	Infections (mainly HCV), autoimmune disorders, lymphoproliferative disorders, or essential (idiopathic)

Abbreviations: HCV, hepatitis C virus; Ig, immunoglobulin; RF, rheumatoid factor.

ness, arthralgia, and, in some patients, organ involvement. On the basis of immunochemical studies, Brouet et al[4] identified 3 types of cryoglobulins. In type I, the cryoprecipitable immunoglobulin is a single monoclonal immunoglobulin, whereas types II and type III cryoglobulinemias are both mixed types (mixed cryoglobulinemia) composed of at least 2 immunoglobulins. In both type II and type III, a polyclonal IgG is bound to another immunoglobulin that is an antiglobulin and acts as a rheumatoid factor. The primary distinction between type II and type III mixed cryoglobulinemias is that the rheumatoid factor in type II (most often of the IgM class) is monoclonal, whereas in type III, it is polyclonal (Table 1). Both components of mixed cryoglobulinemias, IgG and IgM rheumatoid factor, are necessary for precipitation in cooler milieus, whereas the components individually do not have this property.

Patients are considered to have a significant cryoglobulin level when it is >0.05 g/L on 2 determinations. Some laboratories characterize cryoglobulinemia using immunofixation or immunoelectrophoresis and quantify cryoglobulin level by determining cryocrit as a percentage of total volume. Use of immunoblotting for immunochemical characterization is a sensitive and specific method allowing full identification in 98% of cases.[5] When a cryoglobulin is suspected, serum should be kept warm and tests should be carried out at 37°C. Serum cryoglobulins also may lead, by analytical interference, to spurious quantification of plasma proteins and erythrocyte sedimentation rate, pseudo-leukocytosis, pseudo-thrombocytosis, or pseudo-macrocytosis.

Mixed cryoglobulinemias represent 60%-75% of all cryoglobulinemia and are found in connective tissue diseases and infectious or lymphoproliferative disorders; in other words, secondary mixed cryoglobulinemia. After the identification of HCV,[6,7] it has been recognized as the cause of 80%-90% of mixed cryoglobulinemia. In general, HCV is associated with type II mixed cryoglobulinemia (which usually involves an IgM κ molecule having rheumatoid factor function with anti-idiotypic activity), although to a lesser degree, it also is associated with type III mixed cryoglobulinemia.[8-10] In the absence of an identified etiologic factor (<5% of all mixed cryoglobulinemia), cryoglobulinemic vasculitis is defined as essential or idiopathic. This review provides an overview of HCV-induced cryoglobulinemia with a focus on kidney involvement.

EPIDEMIOLOGY

Besides chronic liver disease, relevant but relatively infrequent extrahepatic manifestations of HCV include cryoglobulinemia, lymphoproliferative disorders, and kidney diseases, with descriptions of glomerular manifestations in both native[5] and transplanted kidneys.[11-13] A variety of kidney diseases have been associated with HCV (Table 2). Most notably, when El-Serag et al[14] identified 34,204 hospitalized US male veterans with HCV infection (cases) and 136,816 randomly selected patients without HCV infection (controls) between 1992 and 1999, a significantly greater proportion of HCV-infected patients had porphyria cutanea tarda (0.77% vs 0.06%; $P < 0.0001$), vitiligo (0.17% vs 0.10%; $P = 0.0002$), lichen planus (0.30 vs 0.13; $P < 0.0001$), and cryoglobulinemia (0.57% vs 0.05%; $P < 0.0001$). There was a greater proportion of MPGN in patients with HCV infection (0.36% vs 0.05%; $P < 0.0001$), but not membranous glomerulopathy (0.33% vs 0.19%; $P = 0.86$).

During the last decade, studies using large clinical databases suggest that HCV may influence the incidence (and prevalence) of kidney disease,[15-21] including in the presence of human immunodeficiency virus (HIV) coinfection.[22,23] Although chronic HCV infection is associated with well-defined entities such as mixed cryoglobulinemia and MPGN,[24] whether HCV is associated with abnormal kidney measures in the broader population is unclear (Table 3). For example, 4 cross-sectional surveys have shown a significant link between HCV and proteinuria in apparent healthy individuals (Table 4),[15,20,25,26] and the risk of proteinuria in patients with HIV-HCV coinfection is higher than in those with HIV infection alone.[22] Additionally, other studies have shown that anti-HCV antibody

Table 2. HCV-Associated Kidney Diseases: Primary Pathogenesis and Manifestations

Kidney Disease	Pathogenesis	Clinical Manifestations
Cryoglobulinemic membranoproliferative glomerulonephritis	Mesangial deposits of immune complexes (HCV viral antigens, Ig, and complement fragments); cryoglobulin deposition in glomerular capillaries, mesangium, and urinary space	Nephritic syndrome, nephrotic syndrome
Noncryoglobulinemic membranoproliferative glomerulonephritis	Mesangial deposits of immune complexes (HCV viral antigens, Ig, and complement fragments)	Nephritic syndrome, nephrotic syndrome
Noncryoglobulinemic membranous glomerulopathy	Subepithelial deposits of immune complexes (HCV viral antigens, Ig, and complement fragments)	Nephrotic syndrome
Noncryoglobulinemic IgA nephropathy	Mesangial deposits of immune complexes (HCV viral antigens, Ig, and complements fragments)	Isolated proteinuria and/or hematuria
Noncryoglobulinemic focal segmental glomerulosclerosis	Direct injury by HCV on podocytes of epithelial cells	Nephrotic syndrome, isolated proteinuria
Immunotactoid glomerulopathy fibrillary glomerulonephritis	Mesangial and capillary wall deposition of immune complexes (HCV viral antigens, Ig, and complement fragments)	Nephrotic syndrome, isolated proteinuria and/or hematuria
Mesangial proliferative glomerular nephritis	Direct effect of HCV on mesangium by TLR-3 or MMP-2	Isolated proteinuria and/or hematuria
Tubulointerstitial nephritis	HCV deposition in tubular epithelial and infiltrating cells (direct cytotoxicity and/or immune-mediated injury)	Proteinuria
Thrombotic microangiopathy	Endothelial injury by direct activity of HCV	Nephrotic syndrome, isolated proteinuria and/or hematuria

Abbreviations: HCV, hepatitis C virus; Ig, immunoglobulin; MMP-2, matrix metalloprotease 2; TLR-3, Toll-like receptor 3.

positivity is associated significantly with proteinuria independent of common metabolic factors, such as diabetes mellitus, arterial hypertension, obesity, and dyslipidemia.[26]

Type I MPGN associated with type II mixed cryoglobulinemia remains the form of kidney disease most frequently observed in direct relation to HCV, whereas less commonly seen kidney lesions include MPGN without cryoglobulinemia and membranous nephropathy. On occasion, there have been reports of focal segmental glomerulosclerosis, fibrillary or immunotactoid glomerulopathies, and thrombotic microangiopathy.[5] In addition, vasculitis and interstitial nephritis have been associated with HCV. More recent information has been accumulated for the association between HCV and glomerular disease in liver-[27,28] or kidney/liver-transplant[29] populations. The natural history of these HCV-associated nephropathies is characterized by remission and relapsing phases; however, the long-term outcome is not well known.

Table 3. Association of Anti-HCV Seropositive Status With Low eGFR in Adjusted Analyses

Study	OR (95% CI)	Study Design
Tsui et al[15] (2006)	0.89 (0.49-1.62)	Cross-sectional
Tsui et al[16] (2007)	0.91 (0.88-0.95)	Cross-sectional
Tsui et al[16] (2007)	2.80 (2.43-3.23)	Retrospective cohort
Dalrymple et al[17] (2007)	1.08 (0.88-1.33)	Cross-sectional
Wyatt et al[22] (2008)	1.49 (1.08-2.06)	Meta-analysis of population-based studies
Moe et al[18] (2008)	0.694 (0.62-0.77)	Cross-sectional
Moe et al[18] (2008)	1.024 (0.90-1.15)	Retrospective cohort
Lee et al[20] (2010)	1.30 (1.20-1.42)	Cross-sectional
Asrani et al[19] (2010)	0.90 (0.36-2.27)	Cross-sectional
Asrani et al[19] (2010)	0.92 (0.79-1.08)	Retrospective cohort
Butt et al[21] (2011)	1.30 (1.23-1.37)	Retrospective cohort

Note: All studies were performed in the United States except for Lee et al, which was performed in Taiwan, and Wyatt et al, which is a meta-analysis of studies conducted in a number of different countries/regions (Australia, Europe, France, Germany, Italy, the Netherlands, Portugal, Spain, and the United States).

Abbreviations: CI, confidence interval; eGFR, estimated glomerular filtration rate; HCV, hepatitis C virus; OR, odds ratio.

Table 4. The Association of Anti-HCV Seropositive Status With Proteinuria in Adjusted Analyses

	OR (95% CI)	Patients With Diabetes	Country
Liangpunsakul & Chalasani[25] (2005)	1.99 (1.38-2.85)	1,349 (8.8%)	US
Huang et al[26] (2006)	1.648 (1.246-2.179)	1,241 (12.5%)	Taiwan
Tsui et al[15] (2006)	1.84 (1.0-3.37)	751 (5%)	US
Wyatt et al[22,a] (2008)	1.25 (1.02-1.30)	NA	Various
Lee et al[20] (2010)	1.14 (1.003-1.30)	5,302 (9.6%)	Taiwan

Abbreviations: CI, confidence interval; HCV, hepatitis C virus; NA, not available; OR, odds ratio.
[a]Meta-analysis of population-based studies.

PATHOPHYSIOLOGY OF MIXED CRYOGLOBULINEMIA VASCULITIS

Overview

Cryoglobulinemia vasculitis is a systemic vasculitis that involves mostly small and, with less frequency, medium-sized arteries and veins. In cryoglobulinemia vasculitis, immune complexes comprising rheumatoid factor, IgG, HCV RNA, and complement are deposited on endothelial surfaces, causing vascular inflammation. Mixed cryoglobulinemia also characteristically involves an increase in B-cell clones that produce pathogenic IgM with rheumatoid factor activity and represents a form of immune complex vasculitis (Fig 1). Intravascular cryoglobulin precipitation is induced by cold temperature and may involve primarily the skin, peripheral nerves, and kidney. A leukocytoclastic reaction commonly is involved in vessel damage when cutaneous vasculitis is present. In patients with peripheral neuropathy, nerve pathology often highlights moderate to severe axonal damage (differential fascicular loss of axons' axonal degeneration and indications of demyelinization) associated with vasculitis of small vessels (arterioles, venules, and capillaries) and infiltration by monocytes and T lymphocytes only, without necrotizing angiitis.[30] Morphologic features observed on kidney biopsy specimens from patients with kidney involvement characteristically include monocyte infiltrate with double contours of the basement membrane and large eosino-

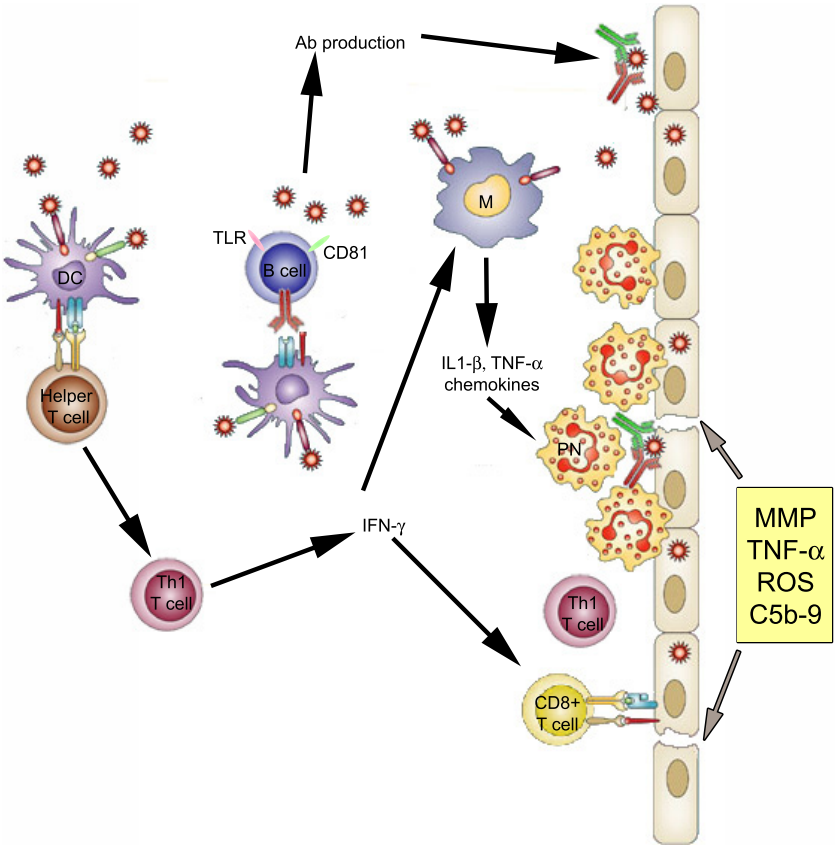

Figure 1. A depiction of the mechanisms that cause hepatitis C virus (HCV)-related mixed cryoglobulinemia vasculitic lesions. HCV associates with the B cell through CD81 and induces persistent stimulation. The B cell then generates antibodies against HCV, which crosslink with immunoglobulin M (IgM) with rheumatoid factor activity and may form the immune complexes known as cryoglobulins. Cryoglobulinemia vasculitis characteristically involves immune complex–mediated tissue injury with deposition of immune complexes, followed by neutrophil recruitment and complement activation. Macrophages (M) and T cells also are present in vasculitic tissues with a predominant T_H1 (T helper cell type 1) type cytokine differentiation. The final stage leading to vasculitis lesions involves activation of the proteolytic activity of matrix metalloprotease (MMP), oxidative stress molecules (ROS), and proinflammatory cytokines such as tumor necrosis factor α (TNF-α) driving the differentiation of dendritic cell (DC) subsets to generate rheumatoid factor. Abbreviations: Ab, antibody; IFN-γ, interferon γ; IL-1β, interleukin 1β; PN, polymorphonuclear neutrophil; TLR, Toll-like receptor.

philic and amorphous intraluminal thrombi, evocative of MPGN. Intraglomerular subendothelial deposits of IgG, IgM (identical to those of the cryoprecipitates), and complement components can be detected by immunofluorescence microscopy. Moreover, one-third of patients have vasculitis of small renal arteries. Extracapillary crescents rarely are observed.[31] Unlike the pattern seen in cutaneous vasculitis, HCV RNA is not prominent in immune complexes in kidney lesions and has not been observed in peripheral neuropathy lesions.

These findings suggest that different pathophysiologic processes are involved in different target organs. In the prevalent pathogenetic mechanism of HCV-associated cryoglobulinemic glomerulonephritis, the virus activates permanent clones of B lymphocytes, which produce monoclonal IgM rheumatoid factor. IgM rheumatoid factor is deposited in the glomerulus, accumulating preferentially in the glomerular matrix. Data from a number of studies suggest that HCV virions may bind directly or indirectly to B cells; however, the virus only infrequently infects B cells.[32-34] Marukian et al[35] have shown that B cells do not express the surface molecules required for HCV entry and thus are incapable of supporting replication of HCV. In a small percentage of cases, immune complexes comprising HCV antigens and anti-HCV IgG antibodies may be deposited directly in glomerular structures; this phenomenon occurs without simultaneous type II mixed cryoglobulinemia with a monoclonal IgM rheumatoid factor, reminiscent of the immune complex glomerulonephritis observed in hepatitis B virus–infected individuals. Such a mechanism could explain the noncryoglobulinemic glomerulonephritis occasionally observed in patients with chronic HCV infection.[36]

Role of HCV and HCV-induced B-Cell Lymphoproliferation

Various pieces of evidence support the etiologic role of HCV in mixed cryoglobulinemia. First, a high proportion of patients with both mixed cryoglobulinemia and chronic liver disease have serologic evidence of HCV infection.[37] Second, HCV RNA is detected in most (up to 90%) patients with type II essential mixed cryoglobulinemia.[38-40] Third, an up to 10-fold increased concentration of anti-HCV IgG is measured in cryoprecipitate.[39] Most of the known HCV antigens (core protein, viral envelope proteins E1 and E2, and nonstructural proteins NS34, NS4, and NS5) and their corresponding antibodies are described in both cryoprecipitate and vascular lesions in tissue sections,[41] and HCV RNA has been found in cryoprecipitate of patients with type II mixed cryoglobulinemia at a concentration up to 1,000 times greater than the level present in supernatant.[39,40]

HCV persistently stimulates the immune system, which may result in the proliferation of B-cell clones producing pathogenic IgM rheumatoid factor. The profound association between this anti-HCV B-cell response and detection of a rheumatoid factor in mixed cryoglobulinemia vasculitis may be explained by the homology (both structural and antigenic) shared between the amino-terminal region of the HCV E2 envelope protein and the human immunoglobulin variable domains. Another possible contributing factor is the interaction of E2 envelope glycoprotein with CD81 B-cell surface protein, which may be strongly stimulatory if binding occurs as a part of a complex (CD19/CD21/CD81) together with B-cell receptor activation.[42] The role of genetic heterogeneity of the hypervariable region of HCV has not been confirmed.[43] Charles and Dustin[42] suggested that certain HCV proteins are needed for expansion of clonal B cells. High concentrations of the HCV envelope protein E2 in vitro trigger expansion of B cells through interaction with CD81, an entry factor for E2.[44] IgG-bound HCV is a specific driver in the expansion of clonal B cells, secreting IgM rheumatoid factor; upon chronic HCV infection, immune-complexed HCV stimulates the expansion of B cells producing rheumatoid factor WA (ie, the variable regions of the immunoglobulin heavy chain are composed of subgroups 1-69 [V_H-1-69$^+$]).[42] These cells become clonally predominant by continued antigenic exposure (usually over a decade or more) and do not require T-cell help.[42] The HCV E2-CD81 interactions could result in a lowered B-cell stimulation threshold, facilitating the secretion of various antibodies, including IgM rheumatoid factor. It still is not understood why clonal B-cell expansion shown in this setting[45,46] appears to be more common in chronic HCV compared with chronic hepatitis B virus or HIV infection. This difference may be related to the observation that serum B-cell activating factor, a member of the tumor necrosis factor α cytokine family necessary for B-cell survival, has been found in HCV-associated mixed cryoglobulinemia.[47,48]

In $<$10% of patients, expansion in monoclonal B cells leading to type II mixed cryoglobulinemia may progress into overt B-cell non-Hodgkin lymphoma. The change from polyclonal B-cell proliferation (type III mixed cryoglobulinemia) to oligo/monoclonal B-cell proliferation (type II mixed cryoglobulinemia) and to malignant lymphoma occurs over many steps, most likely requiring a number of mutagenic events,[49,50] and the duration of B-cell stimulation

caused by infectious or other exogenous agents has been implicated.

Role of Autoantibodies in Cryoglobulinemia Vasculitis

The finding that immunoglobulin molecules and complement fractions are observed in the wall of affected microvessels not showing cellular exudation supports the contention that cryoglobulins have detrimental consequences. In a murine model of cryoglobulin-induced immune complex glomerulonephritis, accumulation of neutrophils was shown to occur through C5 activation.[51] There also is evidence suggesting that faulty processing and reduced clearance of mixed cryoglobulinemia immune complexes favors tissue deposition.[52] Lesions in the skin and glomerulus that are similar to the damage seen in cryoglobulinemic vasculitis have been induced in healthy mice by injecting them with a monoclonal antibody showing both cryoglobulin and rheumatoid factor activities that was obtained from a mouse strain with systemic autoimmunity (MRL-lpr/lpr). Accordingly, although both the rheumatoid factor and cryoglobulin functions of the monoclonal antibody are necessary for skin vasculitis to develop, cryoglobulin activity alone is enough to create glomerular lesions.[53]

Involvement of Cellular Immunity in Cryoglobulinemic Vasculitis

The pathogenesis of HCV-associated mixed cryoglobulinemia vasculitis appears to involve a number of mechanisms. Useful experimental model systems for vasculitis in HCV-related mixed cryoglobulinemia (eg, Arthus and similar models) exist and are characterized histologically by tissue edema, hemorrhage, and neutrophilic infiltration. Experimental data suggest that intradermal administration of IgM rheumatoid factor gives an effective reverse passive cutaneous Arthus reaction and vasculitis.[54] However, a frequent feature of HCV mixed cryoglobulinemia vasculitis is an inflammatory infiltration of lymphocytes and monocytes around small and precapillary arterioles, suggesting T-cell–mediated pathogenesis.[55]

In the last few years, $CD4^{+}CD25^{high}$ immunoregulatory T cells have been identified as major players in autoimmune responses; patients with mixed cryoglobulinemia vasculitis have fewer peripheral-blood Treg (regulatory T) cells than HCV-infected individuals without autoimmune manifestations.[56] A positive correlation has been found between clinical responses and Treg cell levels, suggesting a dual role for Treg cells in the pathogenesis of HCV-induced mixed cryoglobulinemia vasculitis: although Treg cells may hamper the immune system's efforts to eliminate the virus, at the same time they keep autoimmune injury from becoming too severe.[57]

More recently, patients with HCV-related autoimmunity have been shown to have expansion of functionally anergic $CD21^{-/low}$ marginal zone-like B-cell clones,[58] leaving them more vulnerable to the development of autoimmunity and/or lymphoproliferation. Instead of being eliminated, these cells persist in peripheral blood, where the continual antigenic stimulation through Toll-like receptors can create a setting conducive to loss of tolerance and activation of these cells.[59]

Pathogenesis of Kidney Injury in HCV-Induced Mixed Cryoglobulinemia

Some evidence supports the view that kidney injury due to HCV is mediated by cryoglobulins. Cryoglobulins are deposited in the mesangium, where they appear as intense subendothelial IgM deposits by immunofluorescence microscopy. Their nephrotoxicity is related to a special affinity of the IgM-κ rheumatoid factor for cellular fibronectin present in the mesangial matrix.[60,61]

The typical histopathologic lesion of cryoglobulinemic glomerulonephritis is a membranoproliferative (mesangiocapillary) glomerulonephritis.[62] It has been detected in ~80% of patients with HCV-associated type II mixed cryoglobulinemia and glomerular involvement.[63] Eosinophilic thrombi comprising cyroglobulin deposits also may be present, usually associated with vasculitis and fibrinoid necrosis of glomeruli. Endothelial injury may be an expression of the direct cytopathic activity of the virus. In addition, cryoglobulins may trigger endothelitis through antiendothelial antibody activity and complement activation, which in turn may lead to the overexpression of vascular cell adhesion molecule 1 (VCAM-1) and platelet aggregation thereafter.[62,64,65] Immune complexes containing HCV antigens have been observed in the mesangium of patients with cryoglobulinemia, leading to mesangial expansion,[66] and the existence of HCV-related proteins in the mesangium has been linked to greater proteinuria, potentially a reflection of direct mesangial damage by HCV.[62,67]

CLINICAL FEATURES

Clinically, essential mixed cryoglobulinemia is characterized by the triad of purpura, arthralgias, and weakness. The clinical course of patients with mixed cryoglobulinemia is variable: some patients have an indolent course while others develop vasculitic lesions in various organs. Of particular importance is the development of kidney disease because glomerulonephritis is associated with a poor prognosis. Extrarenal features of mixed cryoglobulinemia include neu-

ropathy, hepatomegaly, sicca syndrome, and central nervous system and gut involvement. Peripheral neuropathy is a combined motor and sensory polyneuropathy, mainly distal and of subacute onset. Occasionally, patients may present with multineuropathy features[68-71] and, very rarely, with central nervous system involvement due to cerebral vasculitis.[72] Gastrointestinal manifestations are reported in 7.4% of patients with HCV mixed cryoglobulinemia vasculitis. Abdominal pain, surgical abdomen, and/or intestinal bleeding constitute the main presentation. Patients with gastrointestinal manifestations are known to show more frequent kidney (75% vs 30%; $P = 0.003$) and cardiac (25% vs 2%; $P = 0.006$) involvement and higher cryoglobulin levels (2.2 vs 1.2 g/L; $P = 0.07$).[73] Overt pulmonary involvement is infrequent. Even so, in one study involving routine checks of pulmonary function, findings consistent with interstitial lung involvement were seen in 61% of patients.[74] Individuals with mixed cryoglobulinemia usually show serum positivity for anti-HCV antibodies and have detectable HCV RNA in serum. The level of serum rheumatoid factor, which is detectable in 16%-70% of HCV-positive patients, usually is increased in the setting of HCV mixed cryoglobulinemia, and serum C4 and C1q levels usually are very low.[42]

The clinical syndrome of mixed cryoglobulinemia vasculitis can be associated with both types II and III cryoglobulins. In clinical surveys, patients with type III mixed cryoglobulinemia outnumber those with type II mixed cryoglobulinemia.[75] However, patients with kidney involvement typically have more type II mixed cryoglobulinemia, with the monoclonal IgM component composed mostly of molecules with κ light chain.[76] In the few cases of type III mixed cryoglobulinemia that have kidney involvement, glomerular lesions were variable and nonspecific, whereas in type II mixed cryoglobulinemia (with IgM-κ the monoclonal component), the well-characterized pattern of glomerular disease described previously and described as cryoglobulinemic glomerulonephritis occurs.

Although extrarenal signs of mixed cryoglobulinemia vasculitis usually precede the kidney manifestations, often by years, in 29% of cases, kidney and extrarenal involvement are concurrent.[76] Kidney involvement in mixed cryoglobulinemia occurs in 8%-58% of patients, and in a minority of cases, kidney disease can be the first manifestation of mixed cryoglobulinemia. More than half the patients have proteinuria and/or hematuria only.[77] A "nephritic" syndrome is diagnosed in ~20% of cases. Often both nephrotic proteinuria and active urinary sediment with elevated serum creatinine level are present simultaneously. In ~10% of patients, acute oliguric kidney failure is the first indicator of kidney disease. Hypertension is common and may be severe, affecting >50% of patients at the time of diagnosis. The severity of hypertension may mirror the severity of kidney disease.[77]

Type II mixed cryoglobulinemia is most common in the fourth or fifth decade of life,[78] with disease more frequent in women than men and varying widely by geographic region.[78] Mixed cryoglobulinemia usually is characterized by periods of extrarenal symptoms alternating with periods of quiescence. The exacerbation of extrarenal symptoms often is associated with a flare up of kidney disease, but can occur independently. In many patients, kidney disease shows an indolent course, and kidney failure requiring dialysis is rare (<10%). Patients with cryoglobulinemic nephritis have a poor prognosis, mainly because of a high incidence of infectious, end-stage liver, and cardiovascular diseases.[76]

Roccatello et al[79] evaluated 146 patients with cryoglobulinemic nephritis, of whom 87% (n = 127) were HCV positive, and noted type II cryoglobulins (IgG/IgM-κ) in 74.4% of cases; the rest had type III cryoglobulins. Diffuse MPGN was the most common histologic pattern (83%), and older age, higher serum creatinine level, and greater proteinuria at diagnosis were associated with the development of kidney failure and death. Survival at 10 years was only 30%, with cardiovascular disease the cause of death in >60% of patients. Additional causes of death included infections (10%), liver failure (19%), and neoplasia (3%). An older study by Tarantino et al[77] of 105 patients had different results, with deaths caused by infections (21%) and liver failure (19%) nearly as common as those caused by cardiovascular diseases (29%). This may reflect different antibiotic and immunosuppression use. In a more recent study, 151 consecutive HCV RNA–positive patients with mixed cryoglobulinemia vasculitis were prospectively followed up between 1993 and 2009. Factors predictive of poor prognosis were severe liver fibrosis (hazard ratio [HR], 5.3), as well as heart (HR, 4.2), central nervous system (HR, 2.7), and kidney (HR, 1.9) involvement at baseline. Patients treated with antiviral drugs were observed to have a better prognosis, but use of immunosuppressant agents was associated with a worse prognosis. The 1-, 3-, 5-, and 10-year survival rates (since the time of the mixed cryoglobulinemia diagnosis) were 96%, 86%, 75%, and 63%, respectively.[80]

TREATMENT OF HCV-ASSOCIATED MIXED CRYOGLOBULINEMIA AND HCV-INDUCED GLOMERULONEPHRITIS

The discovery of HCV and a better understanding of the mechanisms of disease has provided the oppor-

Table 5. Clinical Studies Assessing Antiviral Treatment of HCV-Associated Glomerulonephritis

Study	Sustained Virologic Response	Antiviral Therapy
Mazzaro et al[84] (2000)	14% (1/7)	Lymphoblastoid: interferon alfa
Bruchfeld et al[85] (2003)	71% (5/7)	Interferon alfa-2b + ribavirin (n = 4); PEG interferon alfa-2b + ribavirin (n = 2)
Rossi et al[88] (2003)	100% (3/3)	Interferon alfa-2b + ribavirin
Alric et al[24] (2004)	67% (12/18)	Interferon alfa + ribavirin (n = 14); PEG interferon alfa + ribavirin (n = 4)
Saadoun et al[89] (2006)	59% (13/22)	Interferon alfa-2b + ribavirin (n = 12); PEG interferon alfa-2b + ribavirin (n = 10)
Roccatello et al[79] (2007)	11% (6/55)	Interferon alfa (n = 10); interferon alfa + ribavirin (n = 45)
Garini et al[86] (2007)	75% (3/4)	Interferon alfa + ribavirin (n = 2); PEG interferon alfa-2a + ribavirin (n = 2)
Abbas et al[87] (2008)	13% (4/30)	Interferon alfa + ribavirin
Saadoun et al[90] (2010)	40% (4/10)	PEG interferon alfa-2b + ribavirin

Note: Pooled analysis of results listed in the table: odds ratio, 0.42 (95% confidence interval, 0.24-0.61).
Abbreviations: HCV, hepatitis C virus; PEG, pegylated.

tunity to control HCV mixed cryoglobulinemia using targeted approaches: (1) antiviral therapy, motivated by the theory that the underlying infection stimulates the formation of immune complexes and the ensuing vasculitis; (2) B-cell depletion therapy targeting B cells that produce cryoglobulins; and (3) nonspecific immunosuppressive therapy targeting inflammatory cells present in vasculitic lesions.[81-83]

Antiviral Agents

There are no large-scale randomized studies evaluating the use of antiviral medications for the treatment of HCV-induced glomerulonephritis. Accordingly, treatment suggestions are dependent on experiences described in small observational cohorts (Table 5)[24,36,84-90] and extrapolation from studies of HCV infection in populations without kidney involvement.

Early studies of HCV infection without kidney involvement used interferon alfa monotherapy, but the combined regimen (pegylated interferon alfa and ribavirin) superseded monotherapy, and more recently, triple therapy with pegylated interferon alfa, ribavirin, and direct-acting viral agents such as telaprevir or boceprevir has emerged as the optimal treatment for chronic HCV genotype 1.[91,92] To date, there are no clinical data for direct-acting viral agent use in patients with decreased kidney function.[93]

In patients with HCV-mixed cryoglobulinemia, monotherapy with standard interferon alfa treatment 3 times a week has been reported to engender a disappointing response, with a high rate of relapse.[36] In particular, interferon alfa monotherapy was effective in 50%-100% of patients with purpuric skin lesions, but had no benefit on neurologic or kidney involvement. Although viral response to interferon alfa correlates with clinical improvement, in studies in which patients are followed up for enough time to draw meaningful conclusions, most of the responders have viral and clinical relapses after withdrawal of therapy.[94-97] Better results have been seen with interferon alfa and ribavirin combination therapy. In 2 uncontrolled studies,[98,99] combination therapy was associated with improvement in HCV mixed cryoglobulinemia manifestations (cutaneous, 100%; kidney, 50%; and neurologic, 25%-75%). Similarly, in a study of 72 consecutive patients with HCV mixed cryoglobulinemia,[89] combination therapy was associated with a high rate of complete clinical (67.5% vs 56.3%) and viral (62.5% vs 53.1%) response compared with the standard interferon alfa plus ribavirin regimen regardless of HCV genotype and viral load.

Taken in sum, available data for antiviral use in patients with HCV infection suggest kidney benefits, including remission of proteinuria and hematuria and improved kidney function as HCV RNA becomes undetectable in serum and concomitant with a decrease in level of circulating cryoglobulins. Even so, eradication of HCV cannot be achieved consistently, and the clinical benefit of antiviral therapy can be short lived and limited to patients who have low-grade organ involvement.[81-83]

A meta-analysis of controlled clinical trials comparing the efficacy and safety of antiviral versus immunosuppressive therapy (corticosteroids alone or with cyclophosphamide) in patients with HCV-induced glomerulonephritis showed that proteinuria decreased more after antiviral therapy consisting of interferon alfa monotherapy for at least 6 months (odds ratio, 3.86).[100] However, neither treatment regimen significantly improved kidney function. Of note, in all patients with proteinuria reduction, HCV RNA clear-

ance was demonstrated at the end of antiviral therapy.[100]

Antiviral therapy for HCV-associated glomerulonephritis has substantial limitations. First, the impact of antiviral therapy on long-term kidney disease outcomes remains uncertain. Second, although response to antiviral therapy may take weeks or months, rapidly progressing kidney disease may be present and kidney failure can develop before virus clearance can be obtained. Third, interferon alfa may exacerbate proteinuria in some patients with underlying glomerulopathies.[101] Finally, ribavirin use in patients with GFR <50 mL/min/1.73 m^2 is not recommended in some guidelines,[102] although recent, albeit preliminary, data support its cautious use in patients with decreased GFR in a well-monitored setting.[83]

B-Cell Depletion Therapy

There are several studies of the efficacy of rituximab, a monoclonal antibody against CD20, in patients with HCV mixed cryoglobulinemia vasculitis who are resistant to or intolerant of antiviral therapy.[103-107] Rituximab is a chimeric antibody that binds to the B-cell surface antigen CD20 and results in rapid depletion of circulating and tissue B cells. It interferes with synthesis of cryoglobulins and monoclonal IgM.

According to a recent review,[108] complete clinical response after rituximab therapy was reported in 60%-70% of patients for whom prior treatments failed to control vasculitis, with cryoglobulin clearance in one-third of patients. However, a relapse of mixed cryoglobulinemia manifestations was noted in 14 of 36 (39%) patients who had demonstrated clinical response.[108] Patients with HCV-associated glomerulonephritis had a significant decrease in proteinuria (to protein excretion <1.0 g/d) after completion of rituximab therapy without side effects or clinical relapses.[103,104] Given the adverse effects associated with rituximab, including infections, elevated liver function test results, and serum sickness–like reactions (Table 6), further studies are necessary to determine whether it is part of the optimal treatment strategy for mixed cryoglobulinemia.[109-125]

Rituximab probably should not be used as monotherapy because the underlying viral infection still needs to be treated. One prospective controlled trial showed benefits with rituximab plus pegylated interferon alfa-2b/ribavirin combined therapy versus pegylated interferon alfa-2b/ribavirin combined therapy alone in 31 patients with severe HCV-associated mixed cryoglobulinemia vasculitis, with a higher rate of kidney complete response (81% vs 40%) and a good safety profile.[90] A similar finding was noted in the 9-patient kidney disease subgroup in another small study.[121]

One large observational cohort of patients with HCV vasculitis recently described the efficacy and tolerance of rituximab with or without combined antiviral therapy.[126] In this study, rituximab monotherapy induced a clinical complete response (ie, each baseline clinical manifestation improved) in 58% of patients and a partial response (ie, at least half the baseline clinical manifestations improved) in 9% of the cohort members. Patients given rituximab treatment in the absence of antiviral therapy were found to have no change in HCV RNA level, with a nonsignificant increase in alanine aminotransferase levels. Thus, this study's findings are reassuring in regard to the use of rituximab in HCV-infected patients, even without antiviral therapy.

Safety concerns exist. Rituximab has been implicated in the reactivation of HCV after kidney transplantation, with complications including cholestatic hepatitis.[127] Additionally, in rare cases, rituximab may form a complex with IgM-κ rheumatoid factor, leading to accelerated cryoprecipitation and severe systemic reactions.[128] To decrease the risk of this side effect, rituximab should be administered at a dose of 375 mg/m^2 per week for 4 consecutive weeks, with plasma exchange performed prior to rituximab infusion in patients with high baseline values of mixed cryoglobulinemia.[129] The role of plasma exchange is discussed more fully next.

Nonspecific Immunosuppressive Agents

Immunosuppressive agents have been given to patients with mixed cryoglobulinemia with serious life-threatening disease complications such as MPGN and severe neuropathy. Combined therapy with corticosteroids and immunosuppressive agents, for example, treatment using both cyclophosphamide and azathioprine, has been tried while awaiting the generally slow response to antiviral treatments. In one retrospective study, 105 patients were treated for cryoglobulinemic vasculitis–associated kidney disease; 80% of patients received corticosteroids and/or cytotoxic agents, whereas 67% were treated by plasma exchange.[77] Even with this aggressive treatment, only 14% of patients experienced long-term remission of kidney disease and only 49% were alive at 10 years.

In 2 controlled studies, corticosteroids, given either alone or in combination with interferon alfa, did not improve HCV-related vasculitis manifestations.[95,96] In one randomized trial, 1 year of methylprednisolone monotherapy was associated with clinical response in 22% of patients in comparison to a clinical response

Table 6. Safety of Rituximab Therapy in Patients With Mixed Cryoglobulinemia and Kidney Involvement: Literature Review

Study	GN/Total Patients	Reported Side Effects
Zaja et al[107] (2003)	2/15	Thrombosis retinal artery (n = 1), panniculitis (n = 1)
Ghijsels et al[109] (2004)	1	None
Kouloukaki et al[110] (2005)	1	None
Basse et al[111] (2006)	7/7	Cryptococcosis (n = 1), disseminated HSV-2 infection (n = 1)
Quartuccio et al[104] (2006)	5/5	Transient moderate neutropenia (n = 1)
Braun et al[112] (2007)	1	None
Visentini et al[113] (2007)	2/6	Transient increase in HCV viremia (n = 1)
De Vita et al[114] (2007)	8/28	First infusion reaction (n = 3), thrombosis of retinal artery (n = 1), panniculitis (n = 1), sepsis (n = 1), hemorrhagic alveolitis (n = 1), severe transient neutropenia (n = 1), transaminase elevation (n = 2)
Petrarca et al[115] (2007)	2/2	None
Korte et al[116] (2008)	1	None
Roccatello et al[106] (2008)	7/12	None
Saadoun et al[117] (2008)	7/16	Mild adverse effects: anemia (n = 8), fatigue (n = 3), fever (n = 2), depression (n = 1), thrombocytopenia (n = 1)
Ruch et al[118] (2009)	1	None
Petrarca et al[119] (2010)	5/19	None
Saadoun et al[90] (2010)	21/38	Serum sickness–like syndrome (n = 5), varicella zoster virus infection (n = 1), erysipelas (n = 1), *Streptococcus pneumoniae* pneumopathy (n = 1)
Wink et al[120] (2010)	1	None
Dammacco et al[121] (2010)	5/22	Fever (n = 2)
Visentini et al[122] (2011)	13/27	Serum sickness syndrome (n = 1), pneumonia by *Legionella* (n = 1), severe anaphylaxis (n = 1), leg thrombophlebitis (n = 1)
Ferri et al[123] (2011)	38/87	Infusion-related reaction (hypotension, n = 2; urticaria, n = 1; serum sickness–like reaction, n = 1), infection (urinary tract infection, n = 2; pneumonia, n = 1; gangrene, n = 1), mild manifestation (n = 9; neutropenia, hypertransaminasemia, hypogammaglobulinemia)
De Vita et al[124] (2012)	7/28	Infection (n = 1), cardiovascular event (n = 2), liver failure (n = 1), gastrointestinal bleeding (n = 1)
Sneller et al[125] (2012)	4/12	Infusion-related fever (n = 1), viral bronchitis (n = 1), leukopenia (n = 1), thrombocytopenia (n = 1), transaminase elevation (n = 4)

Abbreviations: GN, glomerulonephritis; HCV, hepatitis C virus; HSV-2, herpes simplex virus type 2.

in 66% of patients who received interferon alfa monotherapy and 71% of those who received interferon alfa together with methylprednisolone combination therapy.[96] Corticosteroids given in low doses may reduce minor intermittent signs of inflammation such as arthralgia, but are not effective in the event of major organ involvement or for ongoing control of mixed cryoglobulinemia vasculitis.

Theoretically, plasma exchange should help eliminate the pathogenic cryoglobulins from the circulation, thus avoiding a rebound increase in cryoglobulinemia that often is observed after corticosteroid therapy is discontinued. However, when given with interferon alfa, thrice-weekly plasma exchange did not modify the HCV viral response.[129]

Another alternative treatment for HCV-associated mixed cryoglobulinemia vasculitis is low-dose interleukin 2 (IL-2). Preliminary data suggest that IL-2 induces attenuation of inflammatory signs in patients with HCV-induced vasculitis that is refractory to antiviral therapy, rituximab, or both.[130] No severe adverse event was noted in that report. However, further studies are essential before wider implementation of this treatment strategy.

Therapeutic Guidelines and Perspective

Antiviral therapy with pegylated interferon alfa plus ribavirin is suggested for patients with HCV-mixed cryoglobulinemia whose disease severity and activity is mild to moderate (defined as those who do not have rapidly worsening nephritis, motor neuropathy, or other severe complications; Fig 2). As reported in Box 1, the recommended duration of therapy is 1 year.[83] For patients who present with severe disease (defined as progressive motor neuropathy, worsening kidney disease, and extensive skin disease, including ulcers and distal necrosis), a period of induction with rituximab and/or plasma exchange may be useful before starting HCV antiviral treatment.

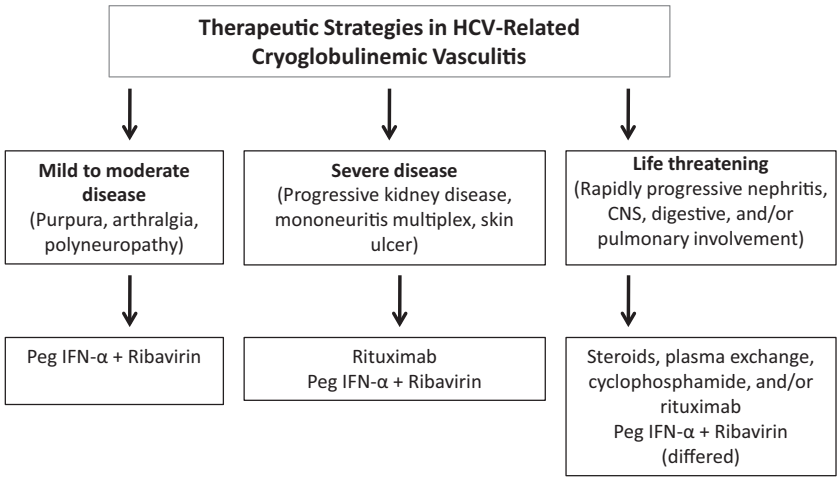

Figure 2. Treatment of hepatitis C virus (HCV)-related mixed cryoglobulinemia vasculitis according to the clinical-biological presentation. Optimal antiviral regimen includes pegylated interferon alfa (Peg IFN-α) plus weight-based ribavirin. According to recent guidelines,[93] the antiviral therapy for genotype 1 chronic HCV is as follows: a DAA (direct-acting antiviral) agent such as boceprevir or telaprevir in combination with Peg IFN-α and ribavirin. If there is failure of the optimal antiviral regimen or a contraindication to antiviral drugs, rituximab may be used alone. Antiviral therapy is recommended in the case of mild to moderate disease including kidney involvement. Abbreviation: CNS, central nervous system.

CONCLUSION

Cryoglobulinemia is a pathologic condition in which the blood contains a group of proteins that are characterized by their tendency to precipitate from cooled serum. As immunocomplexes that deposit on vascular endothelium, cryoglobulins lead to small-vessel vasculitis in various organs, mainly the skin, kidneys, and peripheral nerves. Increasingly, HCV is seen as instigating B-cell lymphoproliferative disorders, including mixed cryoglobulinemia. A well-characterized pattern of glomerular disease termed cryoglobulinemic glomerulonephritis often is seen in individuals with HCV-associated mixed cryoglobulinemia. The most important histologic picture of cryoglobulinemic glomerulonephritis is type I MPGN with subendothelial deposits. Based on a better understanding of pathophysiologic mechanisms, recent advances have been made in the management of HCV-associated cryoglobulinemic vasculitis with kidney involvement. Novel approaches include a modern antiviral regimen, immunosuppressive therapy (rituximab), and plasma exchange, the aggressiveness of which is tailored to the individual presentation.

Box 1. Treatment Strategies for HCV-Induced Cryoglobulinemic Glomerulonephritis

Moderate proteinuria (eGFR ≥60 mL/min/1.73 m^2)
- PEG interferon alfa plus ribavirin (12 mo)
- PEG interferon alfa, ribavirin, and direct-acting antiviral agent (12 mo) for HCV genotype 1
- PEG interferon alfa-2a, 180 μg/wk (subcutaneously)
- PEG interferon alfa-2b, 1.5 μg/kg/wk (subcutaneously)
- Ribavirin, 10-15 mg/kg/d (orally)
- EPO (intravenously/subcutaneously) according to Hb level

Moderate proteinuria and/or stable kidney failure (eGFR ≤60 mL/min/1.73 m^2)
- PEG interferon alfa plus ribavirin (12 mo)
- PEG interferon alfa-2a, 135 μg/wk (subcutaneously)
- PEG interferon alfa-2b, 1.0 μg/kg/wk (subcutaneously)
- Low-dose ribavirin (orally) after implementation of some procedures, including:
  - Weekly monitoring of Hb levels
  - Low ribavirin dose (200-400 mg/d or 200-600 mg for 3 wk)
  - High EPO doses

Nephrotic-range proteinuria and/or rapidly progressive kidney failure
- Phase 1
  - Rituximab intravenously, 375 mg/m^2, per wk for 1 mo
  - Corticosteroid therapy: intravenous methylprednisolone boluses (1.0-0.5 g/d on 3 consecutive days) + oral corticosteroids (0.5 mg/kg/d slowly tapered to 0.1-0.2 mg/kg/d for 4-6 mo)
  - Oral cyclophosphamide (1-2 mg/kg/d for 3-4 mo)
  - Plasma exchange (exchange of 2-3 L of plasma for 2-3 wk)
- After control of vasculitic syndrome has been obtained: phase 2
  - Antiviral therapy (as reported above)

Abbreviations: eGFR, estimated glomerular filtration rate; EPO, erythropoietin; Hb, hemoglobin; HCV, hepatitis C virus; PEG, pegylated.

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