REVIEW ARTICLE

CURRENT CONCEPTS

Edward W. Campion, M.D., *Editor*

Therapy for Hepatitis C Virus–Related Cryoglobulinemic Vasculitis

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CRYOGLOBULINS ARE IMMUNOGLOBULINS CHARACTERIZED BY THEIR insolubility at low temperatures and their dissolution after rewarming.[1,2] On the basis of their immunochemical composition, cryoglobulins are classified as either single (type I), consisting of a monoclonal immunoglobulin, or mixed, comprising two or more immunoglobulin isotypes, with (type II) or without (type III) a monoclonal component.[3] For many years, mixed cryoglobulinemia was referred to as "essential" because of its undefined cause. In the early 1990s, it became evident that more than 90% of patients with mixed cryoglobulinemia were infected with hepatitis C virus (HCV).[4-7] The pathophysiology of mixed cryoglobulinemia, as it is currently understood, is depicted in Figure 1.

Cryoglobulins can be detected in 25 to 30% of HCV-positive patients, but in asymptomatic cases, treatment is unnecessary. Cryoglobulin-related illness, known as cryoglobulinemic vasculitis, appears in a minority (10 to 15%) of patients and includes a spectrum of symptoms (Fig. 2), ranging in severity from mild (sporadic purpura) to life-threatening.[1,2] Consequently, not all patients should be treated to the same extent.[16] This review covers both established treatments for HCV-related cryoglobulinemic vasculitis and emerging therapeutic perspectives (Fig. 3).

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ANTIVIRAL THERAPY

The effectiveness of interferon alfa in the treatment of patients with mixed cryoglobulinemia was observed empirically in 1987[35]; these observations were confirmed and extended in 1991.[36] After it was demonstrated that the large majority of patients with cryoglobulinemic vasculitis are HCV-positive,[4-7] treatment with interferon alfa became a rational therapeutic strategy, yielding clinical, biochemical, and virologic response in 40 to 60% of cases.[37,38] However, this favorable outcome was usually followed by relapse within 6 months after completion of therapy.[38]

The addition of glucocorticoids to interferon alfa therapy did not significantly increase the percentage of patients with a treatment response,[39] although low-to-intermediate doses of glucocorticoids (0.1 to 0.5 mg of prednisone per kilogram of body weight per day), given for weeks or a few months, are believed to mitigate vasculitic flares and to provide symptomatic relief. However, long-term administration of low-dose glucocorticoids (0.1 mg of prednisone per kilogram per day) should be avoided because of their questionable effectiveness and long-term side effects. High doses or pulsed doses of glucocorticoids (1 to 10 mg of prednisone per kilogram) may help to manage severe vasculitic features, including renal and neurologic involvement, and may prevent permanent organ damage.[16]
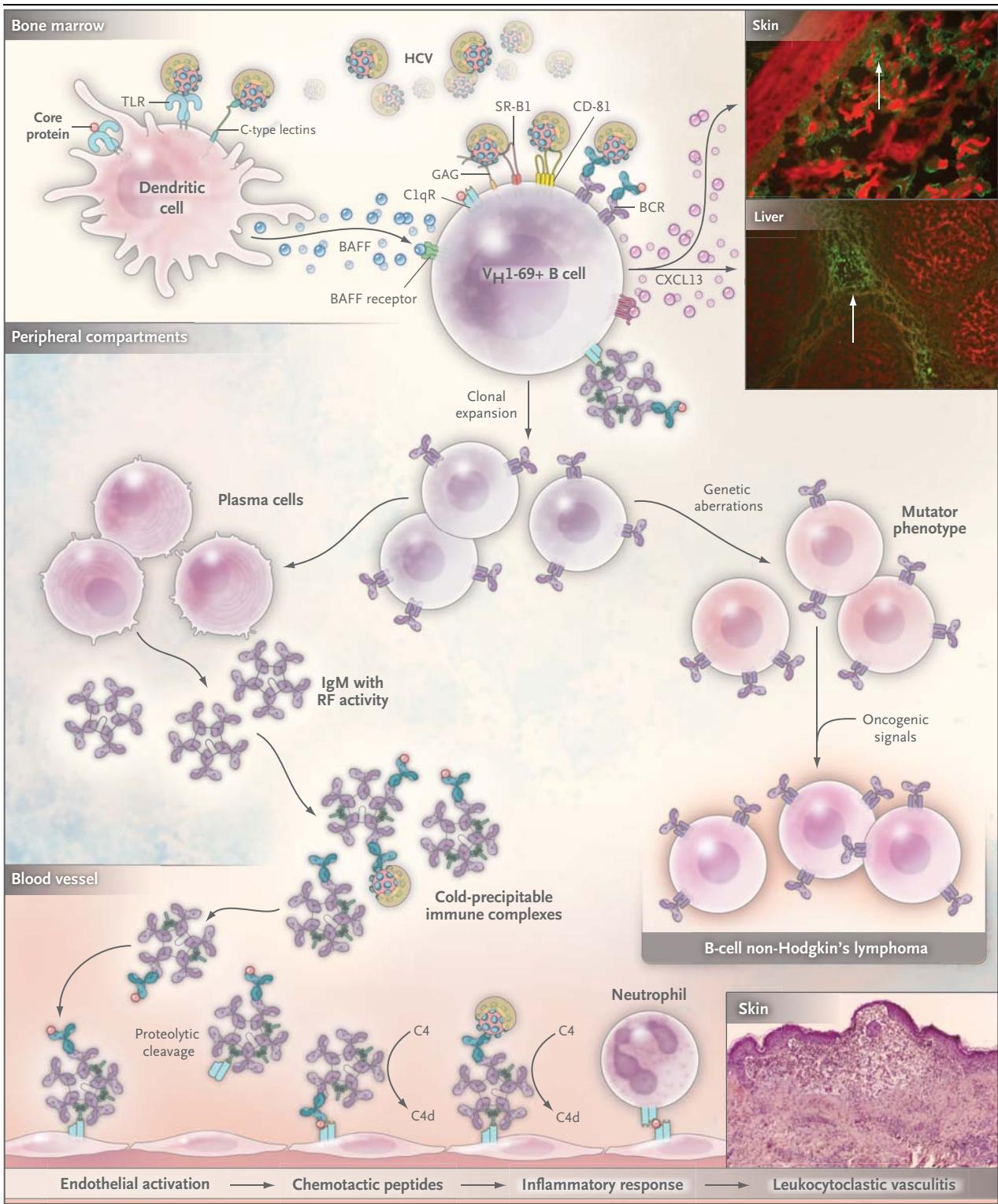
The introduction of once-weekly peginterferon alfa combined with the nucleoside antimetabolite ribavirin as therapy for chronic HCV infection resulted in a

higher number of sustained virologic responses. This combination is now considered one of the standards of care for the management of HCV infection.[40] As expected, the same therapeutic approach was shown to be effective in patients with HCV-related cryoglobulinemic vasculitis. In a preliminary open-label study, treatment with peginterferon alfa and ribavirin resulted in a

Proposed Pathogenetic Model of Hepatitis C Virus (HCV)–Related Cryoglobulinemic Vasculitis.

On their capture of HCV viral particles and HCV core protein through recognition receptors (C-type lectins and toll-like receptors [TLRs]), dendritic cells release B-cell–activating factor (BAFF) of the tumor necrosis factor family. V_H1-69+ B1 cells and marginal-zone B cells capture both HCV viral particles and core protein by means of glycosaminoglycans (GAGs) and the scavenger receptor SR-B1 and CD-81 molecule, in addition to C1q receptor (C1qR) globular domain and B-cell receptor (BCR). These cells are highly responsive to BAFF, which delivers a powerful survival signal through BAFF receptors and plays a key role in sustaining the clonal expansion of B cells[8]; these cells synthesize large amounts of IgM with rheumatoid factor (RF) activity. IgM RF molecules bind HCV viral particles, resulting in the formation of cold-precipitable, multimolecular immune complexes that are good acceptors of C1q protein; this, in turn, leads to their specific binding to endothelial cells through the C1qR. This receptor is proteolytically cleaved, released from the cell surface, and shed in the plasma as a bioactive molecule that circulates as a multiligand protein containing both C1q protein and HCV core protein at two distinct regions of its globular domain.[9] The ensuing complement activation is evidenced by the presence of the C4d fraction. The binding of immune complexes to C1qR globular domain on the surface of endothelial cells generates vasoactive peptides from the complement system and favors the recruitment of inflammatory cells (i.e., neutrophils), which mediate a leukocytoclastic vasculitis. The model also suggests that activated dendritic cells trigger the expression of B-cell chemoattractant CXCL13,[10] which attracts CXCR5+ cells in the skin and liver. The arrows pointing to the immunofluorescence patterns indicate linear deposits of CXCL13 protein (green) along collagen bundles in a skin-biopsy specimen and in a portal tract in a liver-biopsy specimen. The impaired regulatory control of B-cell growth supports an antigen-driven stimulation model in which aberrant B-cell variants potentially evolve into a frank B-cell non-Hodgkin's lymphoma.

complete clinical response and sustained virologic response in 7 of 9 patients (78%).[17] In another open-label study, 86 patients with cryoglobulinemic vasculitis were treated with peginterferon alfa and ribavirin for 6 or 12 months, depending on the HCV genotype.[18] All patients infected with HCV genotype 1 or 4 were given the drug combination for 12 months; only 36% had a sustained virologic response. Of the patients infected with HCV genotype 2 or 3, who underwent 6 months of therapy, 64% had a sustained virologic response. A complete and lasting disappearance of clinical symptoms was recorded in 88% of the patients with a response. Treatment of longer duration (up to 48 weeks for patients infected with HCV genotype 2 or 3 and 72 weeks for patients infected with HCV genotype 1 or 4) has been proposed when improvements in clinical and laboratory measures, but not a complete virologic response, are achieved.[16]

Renal function should be evaluated in all patients before ribavirin is administered for the treatment of cryoglobulinemic vasculitis. Patients with a creatinine clearance of less than 50 ml per minute and those undergoing hemodialysis are not eligible for treatment. Patients with renal impairment should be monitored for the development of hemolytic anemia, and the daily dose of ribavirin should be adapted to the glomerular filtration rate.[41] If the serum creatinine level increases to more than 2.0 mg per deciliter (177 μmol per liter), therapy with peginterferon alfa and ribavirin must be discontinued. Whether ribavirin can be replaced with one or more of the new direct-acting antiviral agents, which do not require dose adjustment in patients with chronic renal disease,[42,43] has not been established.

Several host and viral factors predictive of a poor response or no response to peginterferon alfa and ribavirin have been identified — namely, obesity, hepatic steatosis, insulin resistance, alcohol consumption, drug abuse, advanced age, male sex, African-American ethnic background, high viral load, advanced liver fibrosis, and infection with HCV genotype 1 or 4.[44] Moreover, gene-expression profiling performed on liver-biopsy specimens obtained before therapy has shown the up-regulation of a specific set of interferon-stimulated genes in patients with treatment-resistant disease.[44,45] In addition to the modification of factors susceptible to correction (e.g., obesity, alcohol consumption, and drug abuse), the identification of conditions associated with low response rates qualifies these patients for treatment with the combination of peginterferon alfa, ribavirin, and the new direct-acting antiviral agents, possibly preceded by a 4-week lead-in period of therapy with peginterferon alfa and ribavirin only.

At least a few of the above-mentioned host and viral factors, and probably additional ones, may also influence the response to treatment and account for the poor prognosis, including death, in some patients with cryoglobulinemic vasculitis. Infections, often exacerbated by immunosuppressive treatments, are now considered to be the most common cause of death in patients with severe and rapidly progressive cryoglobulinemic vasculi-
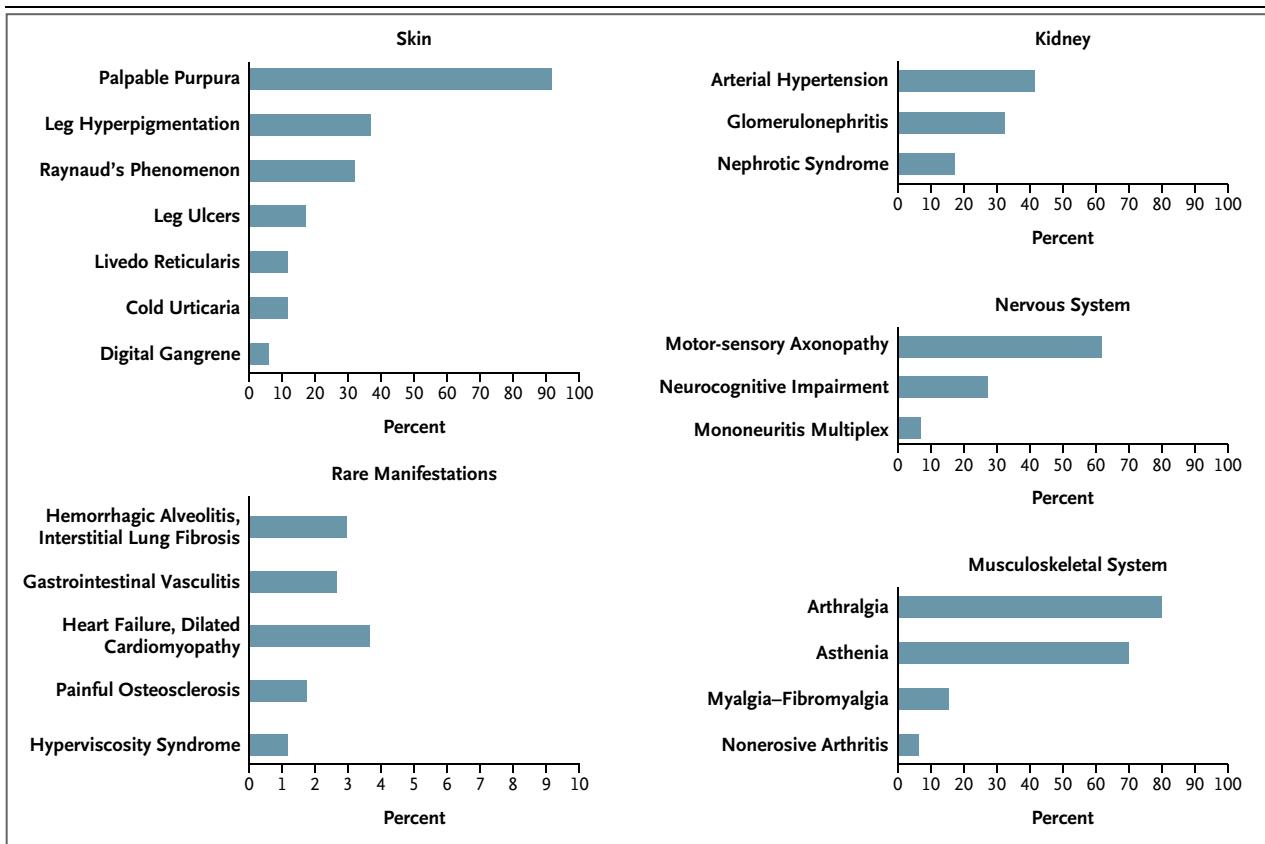
Figure 2. Spectrum of Clinical Features in Patients with HCV-Related Cryoglobulinemic Vasculitis.

The percentages reflect our experience in treating 246 patients with chronic HCV infection and cryoglobulinemic vasculitis. The pathogenesis of rare manifestations is unclear. Hemorrhagic alveolitis may be due to vasculitis that involves small arteries, capillaries, and venules, resulting in interstitial lung fibrosis.[11] Small- and medium-vessel vasculitis accounts for gastrointestinal involvement.[12] Cryoglobulinemic vasculitis–induced cardiomyopathy probably reflects myocardial vessel disease; an association with B-cell non-Hodgkin's lymphoma and severe clinical manifestations has been recognized.[13] HCV-associated osteosclerosis may be caused by an imbalance of the osteoprotegerin–receptor activator of the nuclear factor κB ligand system.[14] Finally, a hyperviscosity syndrome, which is most frequent in type I cryoglobulinemia, develops as a product of the formation of macromolecular cryoprecipitating complexes.[15]

tis. Conversely, the achievement of a sustained virologic response is invariably associated with marked clinical improvement and significantly reduced mortality.[46]

NEW DIRECT-ACTING ANTIVIRAL AGENTS

Antiviral agents that directly target specific viral functions are now available for the treatment of patients infected with HCV genotype 1.[47] Telaprevir and boceprevir have already been approved by the Food and Drug Administration (FDA), and other direct-acting antiviral agents are still under evaluation in phase 1b–3 trials. Telaprevir and boceprevir act as substrates and inhibitors of the hepatic enzyme cytochrome P-450 3A and the drug transporter P-glycoprotein,[48] in addition to acting as HCV NS3/4A protease inhibitors.[49] Triple therapy consisting of telaprevir or boceprevir with peginterferon alfa and ribavirin has markedly increased the rates of sustained virologic response both among patients who have not previously received treatment and among patients who have not had a response to previous treatment regimens. In two phase 3 trials of these triple-therapy regimens in patients infected with HCV genotype 1 who had not previously received treatment, sustained virologic response rates were 68 to 75% — significantly higher than those obtained with peginterferon alfa and ribavirin alone.[50,51] In previously treated patients, however, the extent of prior response to peginterferon alfa and ribavirin was found to strongly influence the effectiveness of triple therapy, with sustained viro-

Figure 3. Drugs for the Treatment of HCV-Related Cryoglobulinemic Vasculitis, According to the Therapeutic Target.
Established drugs are shown in white.[16-21] Drugs for which phase 3 clinical studies or uncontrolled observational data are available are shown in yellow.[22-27] Promising therapeutic agents, the clinical application of which has so far been limited to exploratory studies or must await the results of phase 1–2 studies or of randomized, controlled trials, are shown in pink.[28-34]

logic response rates ranging from approximately 30% among patients with no previous response to 85% among patients with a previous response and subsequent relapse.[48,52-54]

In a preliminary study, 21 patients with chronic HCV genotype 1 infection in whom treatment with peginterferon alfa and ribavirin had failed were randomly assigned to receive 60 mg of daclatasvir (an NS5A replication complex inhibitor) once daily and 600 mg of asunaprevir (an NS3 protease inhibitor) twice daily, either alone (group A) or combined with peginterferon alfa and ribavirin (group B) for 24 weeks.[55] Whereas 36% of patients in group A had a sustained virologic response at 12 and 24 weeks after treatment, all patients in group B had a sustained vi-

rologic response at 12 weeks, and in 90% of them the response was maintained at 24 weeks after treatment. Thus, the administration of the long-established combination of peginterferon alfa and ribavirin together with new direct-acting antiviral agents seems essential for achieving high rates of sustained virologic response. Furthermore, treatment with interferon-sparing regimens or all-oral interferon-free regimens has also been reported to yield genotype-related high rates of sustained virologic response; after treatment completion, response in these trials was evaluated at 12 weeks, as recently approved by the FDA for HCV drug trials, rather than at 24 weeks.[24,25,30]

On the basis of the above-mentioned observations on the effective use of triple therapy in

patients with chronic HCV infection, it was reasonable to anticipate that a similar regimen in patients with HCV-related cryoglobulinemic vasculitis would be equally effective in increasing the likelihood of a sustained virologic response. However, because patients with cryoglobulinemic vasculitis are often older than 50 years of age, anemia was expected to be an important side effect of combining peginterferon alfa and ribavirin with a direct-acting antiviral agent. This topic has been addressed in a recently published article.[22] In an open-label, prospective cohort study, 23 HCV genotype 1–positive patients with cryoglobulinemic vasculitis were treated with the triple-therapy combination of peginterferon alfa and ribavirin with either telaprevir or boceprevir. At week 24, HCV RNA was undetectable in 70% of the patients, and a complete clinical response was achieved in 57% of the patients. In addition, the serum cryoglobulin concentration decreased significantly in conjunction with a significant increase in the C4 complement level. However, a high rate of hematologic side effects (mainly grade 3 and 4 pancytopenia) was recorded. Similar preliminary results have been published in an abstract[23] by another group. A cohort of 35 HCV genotype 1–positive patients with cryoglobulinemic vasculitis and advanced liver disease who had not had a response to therapy with peginterferon alfa and ribavirin were treated with the triple-therapy combination of peginterferon alfa, ribavirin, and boceprevir. Patients were assessed at monthly intervals for a mean duration of 34 weeks. Serum cryoglobulin became undetectable in 79% of these patients, and clinical features markedly improved. Side effects were not specified.

ANTIGEN-DRIVEN B-CELL PROLIFERATION AND THERAPEUTIC DELETION OF B-CELL CLONALITIES

Clonal expansions of IgM rheumatoid factor–producing B cells in HCV-positive patients with mixed cryoglobulinemia have been reported since 1995.[56] HCV RNA genomic sequences have been detected and quantified in intrahepatic B cells isolated from patients with chronic HCV infection, some of whom have had type II mixed cryoglobulinemia.[57] Intrahepatic B cells have been shown to be capable of spontaneously synthesizing rheumatoid factors that display a major cross-reactive idiotype, designated WA.[58] This finding suggests a direct role for HCV in sustaining an in situ

B-cell expansion[59,60] that preferentially involves rheumatoid factor–producing cells.

Consequently, the deletion of B-cell clonalities may provide a rational approach to treatment. It is well known that CD20 antigen, a transmembrane protein, is selectively expressed on pre-B and mature lymphocytes and that CD20-positive cells are expanded and activated in patients with cryoglobulinemic vasculitis.[61-63] Therefore, it was logical to propose the use of rituximab, a chimeric monoclonal antibody specifically directed against CD20 antigen, to treat patients with HCV-related cryoglobulinemic vasculitis that was refractory to conventional antiviral therapy or patients who have had a relapse of disease after therapy.

We evaluated a regimen of four weekly intravenous infusions of rituximab (375 mg per square meter of body-surface area) in 20 patients with HCV-related cryoglobulinemic vasculitis that was resistant to interferon alfa therapy.[19] This strategy resulted in a complete response of clinical and laboratory signs — that is, a reduction in the serum cryoglobulin level and a significant decrease in rheumatoid factor and in anti-HCV antibody titers — in 80% of the patients; in 75% of these patients, the remission was sustained throughout 12 months of follow-up. In addition, a marked depletion or even disappearance of peripheral-blood B-cell clones was observed in the patients with a response. However, rituximab treatment caused a transient increase in HCV RNA levels, whereas levels of alanine aminotransferase were mostly unchanged (Fig. 1S in the Supplementary Appendix, available with the full text of this article at NEJM.org). Although the increased viral load decreased within a few months, and liver enzyme levels were not significantly affected,[64] close monitoring of these measures is warranted.[65]

Zaja et al.[20] administered the above-described rituximab treatment together with medium-to-low doses of glucocorticoids in 12 patients with HCV-related mixed cryoglobulinemia. Clinical symptoms, as well as low-grade non-Hodgkin's lymphoma (occurring in 3 of the patients), significantly improved in most of these patients, and a complete response was achieved in 1 patient with membranoproliferative glomerulonephritis. Furthermore, the serum cryoglobulin level significantly decreased, and the viral load remained stable in most of the patients.

In a randomized clinical trial involving 24 patients with HCV-associated cryoglobulinemic vas-

culitis who had not had a response to treatment with interferon alfa and ribavirin or who had had unacceptable side effects with this treatment, 12 patients were randomly assigned to receive rituximab at the above-mentioned dosage, and the remaining 12 continued to receive maintenance or increased immunosuppressive therapy.[66] At study month 6, a total of 83% of the patients in the group receiving rituximab had a complete response, as compared with 8% in the control group. Both cryoglobulin levels and the viral load decreased significantly in the rituximab group. De Vita et al.[67] conducted a long-term, randomized clinical trial involving 59 patients with cryoglobulinemic vasculitis in whom antiviral therapy had been unsuccessful or was contraindicated. In one group, patients were randomly assigned to receive two 1-g infusions (on days 0 and 14) of rituximab, with retreatment on the same schedule at relapse; the other group received conventional treatment (glucocorticoids, azathioprine or cyclophosphamide, or plasmapheresis). The proportion of patients who continued taking their assigned therapy at 12 months, which was the primary end point of the study, was significantly higher in the rituximab group (64.3%, vs. 3.5% in the conventional-treatment group); the difference remained significant at 24 months. Rituximab also decreased global disease activity. The median duration of the response to rituximab for all three target-organ manifestations (skin ulcers, active glomerulonephritis, and peripheral neuropathy) was 18 months. Changes in the HCV RNA load were not assessed. When patients in the conventional-treatment group who did not have a response were switched to the rituximab group, 61% of them had a response to treatment.

A lower dose of rituximab (250 mg per square meter given twice 1 week apart)[68] was shown to be equally effective. In a study involving 23 HCV-positive patients with cryoglobulinemic vasculitis that was refractory to antiviral therapy, the overall response rate (79%) and side effects were similar to those observed with the higher dosage in the above-described study.[19] Cryoglobulin levels were lowest at month 12 after rituximab administration in patients with a clinical response, and there was no increase in the viral load.

Additional studies[69-72] have addressed the issue of whether rituximab should be used, either alone or in combination with glucocorticoids or antiviral therapy. Because of the marked heterogeneity of these investigations in terms of study design,

HCV genotype, previous treatment, rituximab dose, and concomitant therapy (Table 1S in the Supplementary Appendix), the results are not directly comparable and should therefore be considered with caution. Nevertheless, taken together, the findings support the efficacy, safety, and acceptable side-effect profile of rituximab in the treatment of patients with cryoglobulinemic vasculitis that is resistant to, or that relapses after, antiviral therapy.

In a study assessing the addition of rituximab to peginterferon alfa and ribavirin in order to improve response rates, the triple-drug regimen was administered to 22 HCV-positive patients with cryoglobulinemic vasculitis for 48 weeks; 15 additional patients with the same disease received peginterferon alfa and ribavirin without rituximab.[73] Follow-up proceeded for 36 months after the end of treatment. Among patients treated with the triple-drug regimen, the complete-response rate was 54.5%; among those who received peginterferon alfa plus ribavirin without rituximab, the rate was only 33.3% (P<0.05). Even more interesting were the following observations: in the large majority of patients with a response to the triple-drug regimen (83%), a conversion of B-cell populations from oligoclonal to polyclonal was identified by clonal analysis of B cells from the liver, bone marrow, and peripheral-blood compartments; in all patients who received the triple-drug regimen, the complete response was maintained throughout the follow-up period, whereas this was the case in only 40% of the patients in the control group; and no increase in the viral load was observed in patients who received the triple-drug regimen.

A similar prospective cohort study[74] showed that the addition of rituximab to peginterferon alfa and ribavirin resulted in a shorter time to complete clinical remission, greater efficacy with respect to renal involvement, a higher rate of cryoglobulin disappearance, and more effective clonal B-cell suppression, as compared with peginterferon alfa and ribavirin alone.

Whether rituximab should be administered to patients with cryoglobulinemic vasculitis as first- or second-line therapy remains to be determined. On the basis of clinical evidence from uncontrolled and nonrandomized studies, the combination of peginterferon alfa and ribavirin as first-line therapy is recommended for patients with mild-to-moderate disease activity,[75,76] although peginterferon alfa may exacerbate some of the clinical

features of cryoglobulinemic vasculitis, such as skin ulcers and peripheral neuropathy.[16] However, for patients with active disease that is resistant to the antiviral agents, as well as for patients with life-threatening cryoglobulinemic vasculitis, the addition of rituximab or other B-cell–depleting monoclonal antibodies[26] can restrain the clonal expansion of a rheumatoid factor–producing B-cell subset.[75] This form of triple therapy should be accompanied by plasmapheresis and immunosuppressive therapy. Thus, as a general rule, the choice of the most suitable treatment for a given patient is based strictly on the level of disease activity and the extent and severity of organ involvement[16] (Fig. 4).

PERSISTENT OR RELAPSED CRYOGLOBULINEMIC VASCULITIS AFTER SUCCESSFUL HCV TREATMENT

As the duration of follow-up has increased, a new issue has emerged in the treatment of patients with HCV-related cryoglobulinemic vasculitis — namely, the persistence or relapse of cryoglobulinemic vasculitis in spite of a sustained virologic response to antiviral therapy. Levine et al.[79] described four patients who had cryoglobulinemic vasculitis despite successful eradication of HCV infection with antiviral therapy. Landau et al.[80] reported the clinical features, outcomes, and results of HCV RNA analyses in eight patients with
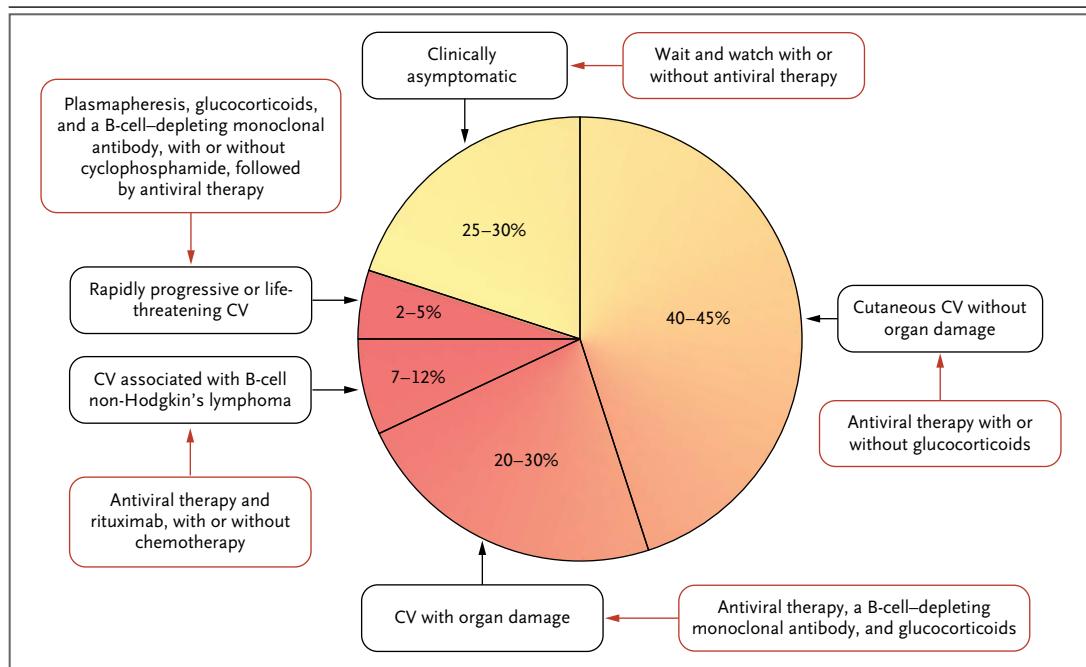

Figure 4. Proposed Therapeutic Algorithm for HCV-Related Cryoglobulinemic Vasculitis (CV), According to Arbitrarily Assessed Disease Activity.

A "wait and watch" strategy is suggested for asymptomatic patients in whom serum cryoglobulins are detected, unless the goal is viral eradication. Antiviral therapy combining peginterferon alfa and ribavirin is advised for patients with clinically symptomatic, mild-to-moderate CV mostly involving the skin. The addition of low-to-medium doses of glucocorticoids may help relieve symptoms. If severe organ damage is present (including nephropathy, peripheral neuropathy, or both), antiviral therapy should be integrated with a B-cell–depleting monoclonal antibody (rituximab or ofatumumab) and glucocorticoids. CV associated with B-cell non-Hodgkin's lymphoma may be treated with a rituximab-containing regimen as first-line therapy, with antiviral agents administered to prevent or reduce the risk of hepatic toxic effects and hepatitis flares.[77] For recurrent or resistant non-Hodgkin's lymphoma, a chemotherapeutic regimen such as fludarabine, rituximab, and cyclophosphamide may be required.[78] Rapidly worsening and life-threatening CV requires prompt therapeutic intervention, initially including a combination of glucocorticoids, plasmapheresis, and a B-cell–depleting monoclonal antibody. If necessary, the short-term administration of an immunosuppressive drug such as cyclophosphamide can be considered, but the risk of infectious complications should be taken into account. Once the emergency phase is over, combination antiviral treatment should be initiated. By analogy with the approach to the treatment of patients with chronic hepatitis C who do not have cryoglobulinemia, a triple-antiviral combination (peginterferon alfa, ribavirin, and either boceprevir or telaprevir) can be adopted to treat patients with HCV genotype 1 infection and patients with refractory or relapsing disease.

HCV-related cryoglobulinemic vasculitis who had a relapse of vasculitis even though they had a sustained virologic response. Transcription-mediated amplification assays for HCV RNA remained consistently negative in both serum samples and cryoprecipitates. Tests for HCV RNA replication in peripheral-blood mononuclear cells were likewise negative. In most of the patients with relapse, the clinical manifestations of cryoglobulinemic vasculitis occurred shortly after discontinuation of antiviral treatment and mainly consisted of purpura and arthralgia, but they either were diminished in intensity or appeared only briefly; in three patients, however, the cryocrit and the clinical manifestations remained unchanged, and a B-cell lymphoma was eventually diagnosed in two of them.

In the typical circulating immune complexes isolated from HCV RNA–positive patients with cryoglobulinemic vasculitis, the virus seems to be the initial inciting agent that triggers the IgG antibody response and, subsequently, the production of a monoclonal IgM component with rheumatoid factor activity.[60] However, circulating immune complexes with a different immunochemical structure also can be envisaged in patients who are still positive for anti-HCV antibodies but have become negative for HCV RNA after antiviral therapy and who continue to have clinical and immunologic features of cryoglobulinemic vasculitis. How should these patients be treated? Since they are negative for HCV RNA, there is no reason to administer antiviral therapy. An obvious alternative would be to use low or medium doses of glucocorticoids, perhaps eventually turning to cyclophosphamide, rituximab, or ofatumumab,[26] a humanized CD20 monoclonal antibody that targets an epitope distinct from the one recognized by rituximab.

ADDITIONAL THERAPEUTIC APPROACHES TO CRYOGLOBULINEMIC VASCULITIS

In addition to the already established dual and triple combinations of antiviral drugs, new categories of direct-acting antiviral agents (Fig. 3) are under preclinical and clinical development. Novel biologic agents are also being developed for the treatment of cryoglobulinemic vasculitis. A short description of these incomplete and rapidly changing therapeutic approaches is provided in the Supplementary Appendix.

DOUBLE-FILTRATION PLASMAPHERESIS

Therapeutic apheresis, a procedure generally performed for palliative purposes, can be useful in the treatment of patients with cryoglobulinemic vasculitis who have a hyperviscosity syndrome.[16] The underlying rationale is the possibility of removing both the cryoprecipitating proteins and the viral particles. Although multicenter, randomized studies comparing plasma exchange with either placebo or immunosuppressive therapy are still lacking,[81] strikingly positive results have been reported.[82,83] In particular, double-filtration plasmapheresis allows the selective removal of pathogenic substances from plasma while preserving other, essential components.[83] In our patients with cryoglobulinemic vasculitis who have inveterate, indolent leg ulcers, double-filtration plasmapheresis has resulted in progressive scarring and eventual complete wound healing. Candidates for double-filtration plasmapheresis are patients with late, refractory-stage cryoglobulinemic vasculitis, as well as those with earlier disease stages. The procedure can be used alone or in combination with antiviral therapy.

CONCLUSIONS

Our understanding of the cause and pathophysiology of HCV-related cryoglobulinemic vasculitis has progressed considerably in the past 15 to 20 years. Although improvements in treatment have also been made, only rarely is disease resolution achieved. The introduction of new antiviral protease inhibitors, new triple-therapy or even quadruple-therapy combinations, and new B-cell–depleting or B-cell–modulating monoclonal antibodies, as well as the possibility of using interferon-free regimens, will expand the panorama of treatment options for chronic HCV infection[84] and, consequently, HCV-related cryoglobulinemic vasculitis, making it possible to individualize the choice of therapy combinations according to the patient's treatment history and HCV genotype.

REFERENCES

1. Dammacco F, Sansonno D, Piccoli C, Tucci FA, Racanelli V. The cryoglobulins: an overview. Eur J Clin Invest 2001;31:628-38.
2. Ramos-Casals M, Stone J, Cid MC, Bosch X. The cryoglobulinaemias. Lancet 2012;379:348-60.
3. Brouet JC, Clauvel JP, Danon F, Klein M, Seligmann M. Biologic and clinical significance of cryoglobulins: a report of 86 cases. Am J Med 1974;57:775-88.
4. Ferri C, Greco F, Longombardo G, et al. Antibodies against hepatitis C virus in mixed cryoglobulinemia patients. Infection 1991;19:417-20.
5. Agnello V, Chung RT, Kaplan LM. A role for hepatitis C virus infection in type II cryoglobulinemia. N Engl J Med 1992; 327:1490-5.
6. Dammacco F, Sansonno D. Antibodies to hepatitis C virus in essential mixed cryoglobulinaemia. Clin Exp Immunol 1992;87:352-6.
7. Misiani R, Bellavita P, Fenili D, et al. Hepatitis C virus infection in patients with essential mixed cryoglobulinemia. Ann Intern Med 1992;117:573-7.
8. Fabris M, Quartuccio L, Sacco S, et al. B-lymphocyte stimulator (BLyS) up-regulation in mixed cryoglobulinaemia syndrome and hepatitis-C virus infection. Rheumatology (Oxford) 2007;46:37-43.
9. Sansonno D, Tucci FA, Ghebrehiwet B, et al. Role of the receptor for the globular domain of C1q protein in the pathogenesis of hepatitis C virus-related cryoglobulin vascular damage. J Immunol 2009;183:6013-20.
10. Sansonno D, Tucci FA, Troiani L, et al. Increased serum levels of the chemokine CXCL13 and up-regulation of its gene expression are distinctive features of HCV-related cryoglobulinemia and correlate with active cutaneous vasculitis. Blood 2008; 112:1620-7.
11. Ferri C. Mixed cryoglobulinemia. Orphanet J Rare Dis 2008;3:25.
12. Terrier B, Saadoun D, Sène D, Scerra S, Musset L, Cacoub P. Presentation and outcome of gastrointestinal involvement in hepatitis C virus-related systemic vasculitis: a case-control study from a single-centre cohort of 163 patients. Gut 2010; 59:1709-15.
13. Terrier B, Karras A, Cluzel P, et al. Presentation and prognosis of cardiac involvement in hepatitis C virus-related vasculitis. Am J Cardiol 2013;111:265-72.
14. Manganelli P, Giuliani N, Fietta P, et al. OPG/RANKL system imbalance in a case of hepatitis C-associated osteosclerosis: the pathogenetic key? Clin Rheumatol 2005;24:296-300.
15. Stone MJ, Bogen SA. Evidence-based focused review of management of hyperviscosity syndrome. Blood 2012;119:2205-8.
16. Pietrogrande M, De Vita S, Zignego AL, et al. Recommendations for the management of mixed cryoglobulinemia syndrome in hepatitis C virus-infected patients. Autoimmun Rev 2011;10:444-54.

17. Cacoub P, Saadoun D, Limal N, Sene D, Lidove O, Piette JC. PEGylated interferon alfa-2b and ribavirin treatment in patients with hepatitis C virus-related systemic vasculitis. Arthritis Rheum 2005;52:911-5.
18. Mazzaro C, Monti G, Saccardo F, et al. Efficacy and safety of peginterferon alfa-2b plus ribavirin for HCV-positive mixed cryoglobulinemia: a multicentre open-label study. Clin Exp Rheumatol 2011;29:933-41.
19. Sansonno D, De Re V, Lauletta G, Tucci FA, Boiocchi M, Dammacco F. Monoclonal antibody treatment of mixed cryoglobulinemia resistant to interferon alpha with an anti-CD20. Blood 2003;101:3818-26.
20. Zaja F, De Vita S, Mazzaro C, et al. Efficacy and safety of rituximab in type II mixed cryoglobulinemia. Blood 2003;101: 3827-34.
21. Thiel J, Peters T, Mas Marques A, Rösler B, Peter HH, Weiner SM. Kinetics of hepatitis C (HCV) viraemia and quasispecies during treatment of HCV associated cryoglobulinaemia with pulse cyclophosphamide. Ann Rheum Dis 2002;61:838-41.
22. Saadoun D, Resche Rigon M, Thibault V, et al. Peg-IFNα/ribavirin/protease inhibitor combination in hepatitis C virus associated mixed cryoglobulinemia vasculitis: results at week 24. Ann Rheum Dis 2013 April 20 (Epub ahead of print).
23. Fabbrizzi A, Monti M, Arena U, et al. Safety and effectiveness of boceprevir triple therapy in patients with mixed cryoglobulinemia. Presented at the 63rd Annual Meeting of the American Association for the Study of the Liver Diseases, Boston, November 9–13, 2012. abstract.
24. Lawitz E, Mangia A, Wyles D, et al. Sofosbuvir for previously untreated chronic hepatitis C infection. N Engl J Med 2013; 368:1878-87.
25. Jacobson IM, Gordon SC, Kowdley KV, et al. Sofosbuvir for hepatitis C genotype 2 or 3 in patients without treatment options. N Engl J Med 2013;368:1867-77.
26. Hagenbeek A, Gadeberg O, Johnson P, et al. First clinical use of ofatumumab, a novel fully human anti-CD20 monoclonal antibody in relapsed or refractory follicular lymphoma: results of a phase 1/2 trial. Blood 2008;111:5486-95.
27. Saadoun D, Rosenzwajg M, Joly F, et al. Regulatory T-cell responses to low-dose interleukin-2 in HCV-induced vasculitis. N Engl J Med 2011;365:2067-77.
28. Vierling JM, Lataillade M, Gane EJ, et al. Sustained virologic response (SVR12) in HCV genotype 1 patients receiving peg-interferon lambda in combination with ribavirin and either daclatasvir or asunaprevir: interim results from the D-LITE Study. Presented at the 63rd Annual Meeting of the American Association for the Study of the Liver Diseases, Boston, November 9–13, 2012. abstract.
29. Zeuzem S, Asselah T, Angus P, et al. Efficacy of the protease inhibitor BI 201335, polymerase inhibitor BI 207127, and ribavi-

rin in patients with chronic HCV infection. Gastroenterology 2011;141:2047-55.
30. Poordad F, Lawitz E, Kowdley KV, et al. Exploratory study of oral combination antiviral therapy for hepatitis C. N Engl J Med 2013;368:45-53.
31. Morschhauser F, Leonard JP, Fayad L, et al. Humanized anti-CD20 antibody, veltuzumab, in refractory/recurrent non-Hodgkin's lymphoma: phase I/II results. J Clin Oncol 2009;27:3346-53.
32. Alduaij W, Ivanov A, Honeychurch J, et al. Novel type II anti-CD20 monoclonal antibody (GA101) evokes homotypic adhesion and actin-dependent, lysosome-mediated cell death in B-cell malignancies. Blood 2011;117:4519-29.
33. Forero-Torres A, de Vos S, Pohlman BL, et al. Results of a phase 1 study of AME-133v (LY2469298), an Fc-engineered humanized monoclonal anti-CD20 antibody, in FcγRIIIa-genotyped patients with previously treated follicular lymphoma. Clin Cancer Res 2012;18:1395-403.
34. Friedberg JW, Vose J, Kahl BS, et al. A phase I study of PRO131921, a novel anti-CD20 monoclonal antibody in patients with relapsed/refractory CD20+ indolent NHL: correlation between clinical response and AUC pharmacokinetics. ASH Annual Meeting Abstracts 2009;114:3472. abstract.
35. Bonomo L, Casato M, Afeltra A, Caccavo D. Treatment of idiopathic mixed cryoglobulinemia with alpha interferon. Am J Med 1987;83:726-30.
36. Casato M, Laganà B, Antonelli G, Dianzani F, Bonomo L. Long-term results of therapy with interferon-alpha for type II essential mixed cryoglobulinemia. Blood 1991;78:3142-7.
37. Ferri C, Marzo E, Longombardo G, et al. Interferon-alpha in mixed cryoglobulinemia patients: a randomized, crossover-controlled trial. Blood 1993;81:1132-6.
38. Misiani R, Bellavita P, Fenili D, et al. Interferon alfa-2a therapy in cryoglobulinemia associated with hepatitis C virus. N Engl J Med 1994;330:751-6.
39. Dammacco F, Sansonno D, Han JH, et al. Natural interferon-alpha versus its combination with 6-methyl-prednisolone in the therapy of type II mixed cryoglobulinemia: a long-term, randomized, controlled study. Blood 1994;84:3336-43.
40. Shepherd J, Brodin H, Cave C, Waugh N, Price A, Gabbay J. Pegylated interferon alpha-2a and -2b in combination with ribavirin in the treatment of chronic hepatitis C: a systematic review and economic evaluation. Health Technol Assess 2004;8:1-125.
41. Perico N, Cattaneo D, Bikbov B, Remuzzi G. Hepatitis C infection and chronic renal diseases. Clin J Am Soc Nephrol 2009;4:207-20.
42. Dumortier J, Guillaud O, Gagnieu MC, et al. Anti-viral triple therapy with telaprevir in haemodialysed HCV patients: Is it feasible? J Clin Virol 2013; 56:146-9.

43. Treitel M, Marbury T, Preston RA, et al. Single-dose pharmacokinetics of boceprevir in subjects with impaired hepatic or renal function. Clin Pharmacokinet 2012;51:619-28.

44. Asselah T, Estrabaud E, Bieche I, et al. Hepatitis C: viral and host factors associated with non-response to pegylated interferon plus ribavirin. Liver Int 2010; 30:1259-69.

45. Chen L, Borozan I, Feld J, et al. Hepatic gene expression discriminates responders and nonresponders in treatment of chronic hepatitis C viral infection. Gastroenterology 2005;128:1437-44.

46. Landau DA, Scerra S, Sene D, Resche-Rigon M, Saadoun D, Cacoub P. Causes and predictive factors of mortality in a cohort of patients with hepatitis C virus-related cryoglobulinemic vasculitis treated with antiviral therapy. J Rheumatol 2010;37:615-21.

47. Pawlotsky JM. New antiviral agents for hepatitis C. F1000 Biol Rep 2012;4:5.

48. Treatment of chronic hepatitis C genotype 1 with triple therapy comprising telaprevir or boceprevir. Swiss Med Wkly 2012;142:w13516.

49. Chatel-Chaix L, Baril M, Lamarre D. Hepatitis C virus NS3/4A protease inhibitors: a light at the end of the tunnel. Viruses 2010;2:1752-65.

50. Poordad F, McCone J Jr, Bacon BR, et al. Boceprevir for untreated chronic HCV genotype 1 infection. N Engl J Med 2011;364:1195-206.

51. Jacobson IM, McHutchison JG, Dusheiko G, et al. Telaprevir for previously untreated chronic hepatitis C virus infection. N Engl J Med 2011;364:2405-16.

52. Bacon BR, Gordon SC, Lawitz E, et al. Boceprevir for previously treated chronic HCV genotype 1 infection. N Engl J Med 2011;364:1207-17.

53. Zeuzem S, Andreone P, Pol S, et al. Telaprevir for retreatment of HCV infection. N Engl J Med 2011;364:2417-28.

54. Ramachandran P, Fraser A, Agarwal K, et al. UK consensus guidelines for the use of the protease inhibitors boceprevir and telaprevir in genotype 1 chronic hepatitis C infected patients. Aliment Pharmacol Ther 2012;35:647-62.

55. Lok AS, Gardiner DF, Lawitz E, et al. Preliminary study of two antiviral agents for hepatitis C genotype N Engl J Med 2012;366:216-24.

56. Franzin F, Efremov DG, Pozzato G, Tulissi P, Batista F, Burrone OR. Clonal B-cell expansions in peripheral blood of HCV-infected patients. Br J Haematol 1995;90:548-52.

57. Sansonno D, De Vita S, Iacobelli AR, Cornacchiulo V, Boiocchi M, Dammacco F. Clonal analysis of intrahepatic B cells from HCV-infected patients with and without mixed cryoglobulinemia. J Immunol 1998;160:3594-601.

58. Gorevic PD, Frangione B. Mixed cryoglobulinemia cross-reactive idiotypes: implications for the relationship of MC to rheumatic and lymphoproliferative diseases. Semin Hematol 1991;28: 79-94.

59. Racanelli V, Sansonno D, Piccoli C, D'Amore FP, Tucci FA, Dammacco F. Molecular characterization of B cell clonal expansions in the liver of chronically hepatitis C virus-infected patients. J Immunol 2001;167:21-9.

60. Sansonno D, Dammacco F. Hepatitis C virus, cryoglobulinaemia, and vasculitis: immune complex relations. Lancet Infect Dis 2005;5:227-36.

61. Vallat L, Benhamou Y, Gutierrez M, et al. Clonal B cell populations in the blood and liver of patients with chronic hepatitis C virus infection. Arthritis Rheum 2004;50:3668-78.

62. Fan HB, Zhu YF, Chen AS, et al. B-cell clonality in the liver of hepatitis C virus-infected patients. World J Gastroenterol 2009;15:1636-40.

63. Charles ED, Brunetti C, Marukian S, et al. Clonal B cells in patients with hepatitis C virus-associated mixed cryoglobulinemia contain an expanded anergic CD21low B-cell subset. Blood 2011;117: 5425-37.

64. Lake-Bakaar G, Dustin L, McKeating J, Newton K, Freeman V, Frost SD. Hepatitis C virus and alanine aminotransferase kinetics following B-lymphocyte depletion with rituximab: evidence for a significant role of humoral immunity in the control of viremia in chronic HCV liver disease. Blood 2007;109:845-6.

65. Nooka A, Shenoy PJ, Sinha R, Lonial S, Flowers CR. Hepatitis C reactivation in patients who have diffuse large B-cell lymphoma treated with rituximab: a case report and review of literature. Clin Lymphoma Myeloma Leuk 2011;11:379-84.

66. Sneller MC, Hu Z, Langford CA. A randomized controlled trial of rituximab following failure of antiviral therapy for hepatitis C virus-associated cryoglobulinemic vasculitis. Arthritis Rheum 2012; 64:835-42.

67. De Vita S, Quartuccio L, Isola M, et al. A randomized, controlled trial of rituximab for treatment of severe cryoglobulinemic vasculitis. Arthritis Rheum 2012; 64:843-53.

68. Visentini M, Ludovisi S, Petrarca A, et al. A phase II, single-arm multicenter study of low-dose rituximab for refractory mixed cryoglobulinemia secondary to hepatitis C virus infection. Autoimmun Rev 2011;10:714-9.

69. Roccatello D, Baldovino S, Rossi D, et al. Long-term effects of anti-CD20 monoclonal antibody treatment of cryoglobulinaemic glomerulonephritis. Nephrol Dial Transplant 2004;19:3054-61.

70. Roccatello D, Baldovino S, Rossi D, et al. Rituximab as a therapeutic tool in severe mixed cryoglobulinemia. Clin Rev Allergy Immunol 2008;34:111-7.

71. Cavallo R, Roccatello D, Menegatti E, Naretto C, Napoli F, Baldovino S. Rituximab in cryoglobulinemic peripheral neuropathy. J Neurol 2009;256:1076-82.

72. Petrarca A, Rigacci L, Caini P, et al. Safety and efficacy of rituximab in patients with hepatitis C virus-related mixed cryoglobulinemia and severe liver disease. Blood 2010;116:335-42.

73. Dammacco F, Tucci FA, Lauletta G, et al. Pegylated interferon-alpha, ribavirin, and rituximab combined therapy of hepatitis C virus-related mixed cryoglobulinemia: a long-term study. Blood 2010;116: 343-53.

74. Saadoun D, Resche Rigon M, Sene D, et al. Rituximab plus Peg-interferon-alpha/ribavirin compared with Peg-interferon-alpha/ribavirin in hepatitis C-related mixed cryoglobulinemia. Blood 2010;116: 326-34.

75. Lamprecht P, Klenerman P. Rituximab in cryoglobulinemic vasculitis: first- or second-line therapy? In: Dammacco F, ed. HCV infection and cryoglobulinemia. New York: Springer, 2012:307-13.

76. St Clair EW. Hepatitis C virus-related cryoglobulinemic vasculitis: emerging trends in therapy. Arthritis Rheum 2012; 64:604-8.

77. Marignani M, Fonzo M, Begini P, et al. 'Les liaisons dangereuses': hepatitis C, rituximab and B-cell non-Hodgkin's lymphomas. World J Gastrointest Pharmacol Ther 2012;3:21-8.

78. Saadoun D, de Chambrun MP, Hermine O, et al. Rituximab plus fludarabine and cyclophosphamide is an effective treatment for refractory mixed cryoglobulinemia associated with lymphoma. Arthritis Care Res (Hoboken) 2012 September 12 (Epub ahead of print).

79. Levine JW, Gota C, Fessler BJ, Calabrese LH, Cooper SM. Persistent cryoglobulinemic vasculitis following successful treatment of hepatitis C virus. J Rheumatol 2005;32:1164-7.

80. Landau DA, Saadoun D, Halfon P, et al. Relapse of hepatitis C virus-associated mixed cryoglobulinemia vasculitis in patients with sustained viral response. Arthritis Rheum 2008;58:604-11.

81. Rockx MA, Clark WF. Plasma exchange for treating cryoglobulinemia: a descriptive analysis. Transfus Apher Sci 2010;42:247-51.

82. Pons-Estel GJ, Salerni GE, Serrano RM, et al. Therapeutic plasma exchange for the management of refractory systemic autoimmune diseases: report of 31 cases and review of the literature. Autoimmun Rev 2011;10:679-84.

83. Ramunni A, Brescia P. Double filtration plasmapheresis: an effective treatment of cryoglobulinemia. In: Dammacco F, ed. HCV infection and cryoglobulinemia. New York: Springer, 2012:337-41.

84. Liang TJ, Ghany MG. Current and future therapies for hepatitis C virus infection. N Engl J Med 2013;368:1907-17.

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