Persistent Hepatitis C Virus—Associated Cryoglobulinemic Glomerulonephritis in Patients Successfully Treated With Direct-Acting Antiviral Therapy

Dominick Santoriello1, Nanda K. Pullela2, Kalpana A. Uday2, Shawn Dhupar3, Jai Radhakrishnan4, Vivette D. D’Agati1 and Glen S Markowitz1

1Department of Pathology and Cell Biology, Columbia University Medical Center, New York, New York, USA; 2Department of Medicine, Division of Nephrology, Bronx Lebanon Hospital Center, Bronx, New York, USA; 3Department of Medicine, Vassar Brothers Medical Center, Poughkeepsie, New York, USA; and 4Department of Medicine, Division of Nephrology, Columbia University Medical Center, New York, New York, USA

Correspondence: Dominick Santoriello, Department of Pathology, Columbia University, College of Physicians and Surgeons, Renal Pathology Laboratory, Room VC14-224, 630 West 168th Street, New York, New York 10032, USA. E-mail: ds3356@cumc.columbia.edu

Kidney Int Rep (2018) 3, 985–990; https://doi.org/10.1016/j.ekir.2018.03.016
© 2018 International Society of Nephrology. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

INTRODUCTION

Recent estimates suggest that 2.7 million to 3.9 million Americans are infected with hepatitis C virus (HCV). Mixed (type II) cryoglobulinemia can be detected in 25% to 30% of patients with chronic HCV, a minority (10%–15%) of whom develop symptoms of cryoglobulinemic vasculitis (CryoVas).1 The pathogenesis of HCV-CryoVas involves viral-induced expansion of B-cell clones that produce IgM with rheumatoid factor (RF) activity. Monoclonal IgM binds to polyclonal IgG that recognizes hepatitis C nucleocapsid and core antigens. The resulting circulating immune complexes deposit in vascular beds, leading to complement activation, leukocyte recruitment, and, ultimately, glomerulonephritis or vasculitis.2 HCV-associated cryoglobulinemic glomerulonephritis (HCV-CryoGN) occurs in 10% to 35% of patients with CryoVas.1

Viral eradication with pegylated interferon-α (IFN) and ribavirin was the mainstay of CryoVas therapy for many years1 and showed variable efficacy in treating CryoGN.3–7 However, in addition to the larger problem of viral resistance, persistent and/or recurrent HCV-CryoVas occurred in a small subset of patients with sustained virologic response (SVR).8,9 B-cell depletion with rituximab provided added benefit in patients with CryoGN in whom viral eradication failed to induce clinical remission.10,11 With the advent of IFN-free direct-acting antiviral (DAA) therapies, SVR rates now exceed 98%, with the added benefit of more favorable dosing schedules and side effect profiles.12 DAAs also appear to be more effective in inducing SVR in the setting of CryoVas than their IFN-based predecessors.13 Despite the fact that DAAs lack the immunomodulatory effects of IFN, they have shown promising results in the treatment of CryoVas and CryoGN and, in some cohorts, have even been associated with reduced use of immunosuppressive therapy.13–18 Although cost concerns have limited broader use, multiple national and international associations have now established CryoVas as a priority indication for DAA therapy.19,20

Reports of HCV-CryoGN despite SVR after DAA therapy are rare. Sollima et al. reported a series that included 5 patients with CryoGN, only 1 of whom achieved a clinical response despite achieving SVR.21 Ghosn et al. reported 2 patients with newly diagnosed HVC-CryoGN on kidney biopsy approximately 1 year after achieving SVR (1 patient with simeprevir and 1 patient with pegylated INF-α, ribavirin, and sofosbuvir).22 Chowdhury and Tsen described a patient with recurrent HCV-CryoGN diagnosed approximately 2 months after achieving SVR with sofosbuvir and ribavirin.23 We report herein 3 cases of biopsy-proven HCV-CryoGN diagnosed in patients who previously had achieved SVR with DAA therapy.

CASE PRESENTATION

Clinical Characteristics

Baseline clinical characteristics prior to DAA therapy are provided in Table 1. All patients had a known history of HCV infection, including 2 patients with genotype 1B and 1 patient with genotype 1A. Two
Table 1. Clinical characteristics of case patients prior to DAA therapy

| Characteristic | Patient 1 | Patient 2 | Patient 3 |
|---------------|-----------|-----------|-----------|
| Age (yr), sex | 58, female | 68, male  | 61, male  |
| Year of HCV diagnosis | 2015 | 1998 | 2004 |
| HCV genotype | 1A | 1B | 1B |
| Prior HCV therapy | No | PEG-INF + RBV | PEG-INF + RBV |
| HCV viral load by PCR (IU/ml) | $3.6 \times 10^6$ | $3.2 \times 10^6$ | $1.1 \times 10^6$ |
| Extrarenal symptoms | None | Arthralgias | None |
| Cryocrit (type) | Negative | 2% (type II) | 5% (type II) |
| Low C4 | Yes | Yes | Yes |
| Positive RF | Yes | Yes | Yes |
| Serum M-spike | No | Yes (IgM-$\kappa$) | Yes (IgM-$\kappa$) |
| SCr (mg/dl) | 0.7 | 2.9 | 2.6 |
| UPCR prior (g/g) | 5.4 | 3.0 | 11 |
| Hematuria | Yes | Yes | Yes |
| Prior kidney biopsy | No | No | Yes (04/2014, MPGN) |

DAA, direct-acting antiviral; HCV, hepatitis C virus; MPGN, membranoproliferative glomerulonephritis; PEG-INF, pegylated interferon; PCR, polymerase chain reaction; RBV, ribavirin; RF, rheumatoid factor; SCr, serum creatinine; UPCR, urine protein to creatinine ratio.

Table 2. Clinical characteristics after DAA therapy at time of kidney biopsy

| Characteristic | Patient 1 | Patient 2 | Patient 3 |
|---------------|-----------|-----------|-----------|
| DAA therapy | Ledipasvir + sofosbuvir | Ledipasvir + sofosbuvir | Ledipasvir + sofosbuvir |
| Interval (mo) between completion of DAA therapy and renal biopsy | 2 | 5 | 10 |
| In SVR? | Yes | Yes | Yes |
| Duration of SVR (d) | 38 | 154 | 318 |
| SCr (mg/dl) | 1.2 | 4.4 | 2.8 |
| UPCR (g/g) | 3.6 | 3.5 | 9.0 |
| Hematuria | Yes | Yes | Yes |
| Extrarenal symptoms | No | No | No |
| Cryocrit (type) | Negative | 3% (type II) | 1% (type II) |
| Low C4 | No | Yes | Yes |
| Positive RF | No | Yes | Yes |
| Serum M-spike | No | Yes (IgM-$\kappa$) | Yes (IgM-$\kappa$) |

DAA, direct-acting antiviral; RF, rheumatoid factor; SCr, serum creatinine; SVR, sustained virologic response; UPCR, urine protein to creatinine ratio.

Pathology

A summary of the renal biopsy findings is provided in Table 3. All 3 patients exhibited a membranoproliferative pattern of glomerulonephritis (MPGN) characterized by mesangial expansion by increased cells and matrix and duplication of the glomerular basement membrane with cellular interposition (Figure 1). Glomeruli also exhibited mild endocapillary proliferation with infiltrating mononuclear leukocytes and rare neutrophils. Interestingly, large, intracapillary immune thrombi typical of HCV-CryoGN were inconspicuous or absent. The degree of tubular atrophy and interstitial fibrosis varied from mild (2 biopsy samples) to moderate (1 biopsy sample). One biopsy sample showed focal endarteritis. By immunofluorescence, all 3 biopsy samples showed IgM-$\kappa$–dominant staining, with weaker-intensity staining for IgG and $\lambda$, characteristic of the type II cryoglobulin deposits seen in the setting of HCV infection. Electron microscopy confirmed the presence of relatively sparse mesangial and subendothelial electron dense deposits, which appeared granular and lacked an organized annular tubular substructure. One patient (patient 3) had a kidney biopsy prior to DAA therapy. Notably, both the intensity of staining by immunofluorescence and the burden of detectable or detectable serum cryoglobulin (2/2). An IgM-$\kappa$ M-spike remained detectable in patients 2 and 3, prompting subsequent hematologic–oncologic evaluation for overt B-cell lymphoma, which was negative in patient 2. A small (<1%) monoclonal B-cell population was identified by flow cytometry performed on bone marrow aspirate in patient 3; however, no overt lymphoma was detected.
immune deposits at the ultrastructural level had decreased on the kidney biopsy performed after completion of DAA therapy.

The biopsy sample from patient 2 was notable for the presence of an atypical B-cell interstitial infiltrate with lymphoplasmacytic differentiation involving <10% of the cortical parenchyma. By immunohistochemistry, the cells were PAX5 positive and aberrantly expressed bcl-2. In situ hybridization highlighted an excess of Κ-positive cells (Κ:Λ ratio, 6:1). Ig heavy-chain gene rearrangement studies performed by fluorescent polymerase chain reaction confirmed the presence of a clonal lymphoid population within the renal parenchyma.

### DISCUSSION

DAAs are highly effective in the treatment of HCV infection, leading to SVR in the overwhelming majority of patients, but are less consistent in treating CryoVas, with rates of clinical response ranging from 64% to 96% and rates of immunological response (defined by the disappearance or marked reduction of circulating cryoglobulins and normalization of RF and C4 levels) ranging from 48% to 89%. Herein we report 3

**Table 3. Pathology findings**

| Finding                        | Patient 1 | Patient 2 | Patient 3 |
|--------------------------------|-----------|-----------|-----------|
| Total glomeruli/GS glomeruli   | 36/1      | 22/5      | 16/4      |
| Primary LM pattern             | MPGN      | MPGN      | MPGN      |
| Glomerular monocyte infiltration| Mild      | Mild      | Mild      |
| Cellular/fibrocellular crescents| 2         | 0         | 0         |
| Segmental scars                | 1         | 0         | 4         |
| Immune thrombi                 | No        | Rare      | No        |
| TAIF (%)                       | 20        | 25        | 40        |
| Endovascularitits              | No        | Yes       | No        |
| Immunofluorescence             |           |           |           |
| IgG                            | 1+        | Trace     | 1+        |
| IgM                            | 2+        | 1+        | 2+        |
| IgA                            | Negative  | Trace     | Negative  |
| C3                             | 2+        | 1+        | 2+        |
| C1                             | 2+        | Negative  | 1+        |
| Kappa (κ)                      | 2+        | 1+        | 2+        |
| Lambda (λ)                     | 1+        | Trace     | 1+        |
| Electron microscopy            | Mes, subendo | Mes, subendo | Mes, subendo |
| Annular-tubular substructure   | No        | No        | No        |
| Foot process effacement (%)    | 50        | 60        | 95        |
| Other findings                 | None      | Clonal B-cell infiltrate | None |

GS, globally sclerotic; MPGN, membranoproliferative glomerulonephritis; Mes, mesangial; subendo, subendothelial; TAIF, tubular atrophy and interstitial fibrosis.

**Figure 1.** (a) Patient 2 underwent kidney biopsy 5 months after completion of direct-acting antiviral (DAA) therapy. Glomeruli were hyperlobulated owing to increased mesangial cells and matrix, thickening and duplication of glomerular basement membranes, and segmental endocapillary leukocyte infiltration, consistent with a membranoproliferative pattern of glomerulonephritis (hematoxylin and eosin, original magnification ×210). (b) Ten months after completion of DAA therapy, and following a course of treatment with rituximab, patient 2 underwent a second kidney biopsy. Glomeruli exhibited a mild increase in the mesangial matrix, correlating with the history of hypertension and tobacco use, but were normocellular, consistent with the resolution of active glomerulonephritis (period acid–Schiff, original magnification ×210).
patients with HCV-CryoGN diagnosed on kidney biopsy after successful completion of DAA therapy, 1 of whom lacked evidence of persistent immune activation but had stable renal disease (patient 1), and 2 of whom had evidence of persistent immune activation and either worsening (patient 2) or stable (patient 3) renal disease. Others have previously reported that persistent (n = 6), recurrent (n = 1), and even de novo (n = 2) HCV-CryoGN can occur despite achievement of SVR.21–23 HCV-CryoGN in the setting of SVR may be an indication for immunosuppressive therapy.

The pathogenesis of HCV-CryoGN involves a number of disturbances in immune homeostasis, particularly the HCV-driven expansion of memory B-cell clones that are responsible for the production of pathogenic IgM with RF activity.13,24,25 Restoration of peripheral B-cell and T-cell homeostasis appears to be an important factor in achieving clinical remission of HCV-CryoVas and Cryo-GN. Patients who achieve a clinical response with DAA therapy alone have decreased proportions of autoreactive memory B-cell clones, as well as increased numbers of Treg cells and decreased numbers of pro-inflammatory Th1 and Th17 subsets.25 Clinical evidence of immunological response (characterized by the disappearance or marked reduction of circulating cryoglobulins and normalization of RF and C4) also appears to correlate with clinical improvement, and may help to stratify patients at risk for persistent CryoGN despite achieving SVR.14

Some studies have shown, however, that immunological improvement may lag behind SVR in the first 12 weeks after DAA therapy.16,26 Therefore, at least in the early posttreatment phase, longer follow-up may result in higher immunological and clinical response rates in some patients, obviating the need for immunosuppressive therapy. This is illustrated by the clinical course of patient 1, who underwent kidney biopsy only 38 days prior to DAA therapy. The duration of sustained virologic response (SVR) at the time of kidney biopsy was 38 days, and at last known follow-up, the patient had stable renal disease with a serum creatinine of 0.7 mg/dl and a urine protein to creatinine ratio (UPCR) of 0.13 g/g. The key event between SVR and kidney biopsy was 38 days.

Figure 2. For each of our 3 patients, serum creatinine and urine protein to creatinine ratio (UPCR) are provided at 3 time points: last known values prior to direct-acting antiviral (DAA) therapy, at the time of kidney biopsy, and at last known follow-up. Also indicated in the key is the duration of sustained virologic response (SVR) at the time of kidney biopsy and the duration of follow-up. HD, hemodialysis.

| Treatment/follow-up | Patient 1 | Patient 2 | Patient 3 |
|---------------------|-----------|-----------|-----------|
| Immunosuppressive therapy | None | RTX | Steroids + RTX + PLEX |
| Interval (mo) between kidney biopsy and last follow-up | 11 | 10 | 23 |
| SCr (mg/dl) | 0.7 | 2.5 | 4.7 (HD-dependent) |
| UPCR (g/g) | 0.13 | 0.5 | 4 |
| Hematuria | No | No | N/A |
| Extrarenal symptoms | No | No | No |
| Cryocrit (type) | Negative | Negative | N/A |
| Low C4 | No | No | N/A |
| Positive RF | Negative | Negative | N/A |
| Serum M-spike | No | No | N/A |

HD, hemodialysis; N/A, not available; PLEX, plasma exchange; RF, rheumatoid factor; RTX, rituximab; SCr, serum creatinine; UPCR, urine protein to creatinine ratio.
after achieving SVR and lacked evidence of persistent immune activation, and subsequently achieved a complete renal response without requiring immunosuppressive therapy. As such, kidney biopsy may not be necessary in the early posttreatment phase in the absence of rapidly worsening renal indices, and may even lead to unnecessary use of immunosuppression. The other 2 patients, however, showed evidence of persistent immune activation at the time of kidney biopsy, and had clinical and pathologic evidence of severe CryoGN 154 and 318 days after achieving SVR, respectively. Therefore, markers of persistent immune activation later in the posttreatment period appear to correlate with ongoing clinical activity and should prompt kidney biopsy in patients with evidence of renal disease.

Persistent immunological and clinical activity presumably is due to persistence of the RF-producing memory B-cell clones, which have been shown to persist for at least 24 weeks in some patients successfully treated with DAAs. In the pre-DAA era, it was shown that resolution of CryoVas after viral eradication was associated with regression of these RF-producing B-cell clones, and that B-cell depletion with rituximab provided added benefit in patients with HCV-CryoVas in whom antiviral therapies alone failed to induce clinical remission. DAAs have been shown to be effective in reducing the frequency of RF-producing B-cell clones in the peripheral blood of HCV-infected patients; however, monoclonal populations can persist after viral eradication. It is interesting to note that the 2 patients (patents 2 and 3) with evidence of immunologic and clinical activity both had IgM-K M-spikes in the serum and identifiable monoclonal B-cell populations (in the renal parenchyma and bone marrow, respectively). Given the lack of evidence of overt lymphoma in these patients, these could be considered as “monoclonal B-cell proliferations of renal significance.” Patient 2 responded well to rituximab, highlighting the utility of B-cell–depleting therapy in cases of refractory or de novo CryoGN in the setting of SVR.

Additional factors lend complexity to the relationship between DAAs, HCV SVR, and persistent HCV CryoGN. Renal response can lag behind viral clearance due to impaired clearance of cryoglobulin-containing immune complexes, particularly in cirrhotic patients. Multiple studies have demonstrated that HCV-RNA levels in the cryoprecipitate can be up to 100- to 1000-fold higher than in the serum and, in rare cases, may result in false-negative serum polymerase chain reaction for HCV RNA. HCV viral particles were detected in the cryoprecipitate of the patient who developed recurrent HCV-CryoGN 2 months after achieving SVR, reported by Chowdhury and Tsen.

This is not a standard laboratory study and was not performed in any of our patients. Therefore, qualitative polymerase chain reaction for HCV-RNA on the cryoprecipitate may be worthwhile in this setting to exclude the much less likely possibility of minimal residual viral replication.

In conclusion, nephrologists and renal pathologists should be aware that HCV-CryoGN can occur in the absence of ongoing viral replication due to persistence of RF-producing memory B-cell clones (Table 5). Evidence of renal disease in the setting of persistent immunological activity (defined by the detectable circulating cryoglobulins, elevated RF levels, and/or low C4 levels), even when associated with SVR, should prompt kidney biopsy to assess the need for immunosuppressive therapy. Future studies may help to determine whether longer follow-up will increase immunological and clinical response rates in patients who receive DAA therapy alone, particularly those with renal involvement. Earlier initiation of DAAs, before the occurrence of severe organ damage, may also improve clinical outcomes, and may even eliminate or reduce the need for immunosuppression in some patients.

Table 5. Teaching points

- The pathogenesis of hepatitis C virus (HCV)–associated cryoglobulinemic glomerulonephritis (HCV-CryoGN) involves the HCV-driven expansion of memory B-cells clones that are responsible for the production of pathogenic monoclonal IgM with rheumatoid factor (RF) activity. Patients who achieve sustained virologic response (SVR) with direct-acting antiviral (DAA) therapy have decreased proportions of these autoantibody-producing memory B-cell clones.
- In a subset of patients, HCV-CryoGN can persist despite achievement of SVR (i.e., in the absence of detectable HCV viremia), likely due to residual RF-producing B-cell clones.
- Clinical evidence of continued immunological activity, characterized by persistent circulating cryoglobulins, RF seropositivity, and/or low C4, may help to stratify patients who are at risk for persistent CryoGN despite achieving SVR.
- B-cell depletion with rituximab may provide added benefit in patients with HCV-CryoGN in whom antiviral therapies alone fail to induce clinical remission.

DISCLOSURE

All the authors declared no competing interests.

REFERENCES

1. Dammacco F, Sansonno D. Therapy for hepatitis C virus-related cryoglobulinemic vasculitis. N Engl J Med. 2013;369:1035–1045.
2. Charles ED, Dustin LB. Hepatitis C virus-induced cryoglobulinemia. Kidney Int. 2009;76:818–824.
3. Gragnani L, Fognani E, Piluso A, et al. Long-term effect of HCV eradication in patients with mixed cryoglobulinemia: a prospective, controlled, open-label, cohort study. Hepatology. 2015;61:1146–1153.
4. Alric L, Plaisier E, Thebault S, et al. Influence of antiviral therapy in hepatitis C virus-associated cryoglobulinemic MPGN. Am J Kidney Dis. 2004;43:617–623.
5. Johnson RJ, Gretch DR, Yamabe H, et al. Membranoproliferative glomerulonephritis associated with hepatitis C virus infection. N Engl J Med. 1993;328:465–470.
6. Rossi P, Bertani T, Baio P, et al. Hepatitis C virus-related cryoglobulinemic glomerulonephritis: long-term remission after antiviral therapy. *Kidney Int. 2003;63:2236–2241.

7. Mahale P, Engels EA, Li R, et al. The effect of sustained virological response on the risk of extrahepatic manifestations of hepatitis C virus infection. *Gut. 2018;67:553–561.

8. Landau DA, Saadoun D, Halfon P, et al. Relapse of hepatitis C virus-associated mixed cryoglobulinemia in patients with sustained viral response. *Arthritis Rheum. 2008;58:604–611.

9. Levine JW, Gota C, Fessler BJ, et al. Persistent cryoglobulinemic vasculitis following successful treatment of hepatitis C virus. *J Rheumatol. 2005;32:1164–1167.

10. 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;116:343–353.

11. Saadoun D, Resche Rigon M, Sene D, et al. Rituximab plus Peg-interferon-alpha/ribavirin in hepatitis C-related mixed cryoglobulinemia. *Blood. 2010;116:326–334; quiz 504–505.

12. Afadh N, Zeuzem S, Kwo P, et al. Ledipasvir and sofosbuvir for untreated genotype 1 infection. *N Engl J Med. 2014;370:1899–1908.

13. Cacoub P, Vautier M, Desbois AC, et al. Effectiveness and cost of hepatitis C virus cryoglobulinaemia vasculitis treatment: from interferon-based to direct-acting antivirals era. *Liver Int. 2017;37:1805–1813.

14. Bonacci M, Sensi L, Londono MC, et al. Virologic, clinical, and immune response outcomes of patients with hepatitis C virus-associated cryoglobulinemia treated with direct-acting antivirals. *Clin Gastroenterol Hepatol. 2017;15:575–583.

15. Saadoun D, Thibault V, Si Ahmed SN, et al. Sofosbuvir plus ribavirin for hepatitis C virus-associated cryoglobulinaemia vasculitis: VASCUVALDIC study. *Ann Rheum Dis. 2016;75:1777–1782.

16. Lauletta G, Russi S, Pavone F, et al. Direct-acting antiviral agents in the therapy of hepatitis C virus-related mixed cryoglobulinemia: a single-centre experience. *Arthritis Res Ther. 2017;19:74.

17. Saadoun D, Pol S, Ferfar Y, et al. Efficacy and safety of sofosbuvir plus daclatasvir for treatment of HCV-associated cryoglobulinemia vasculitis. *Gastroenterology. 2017;153:49–52.

18. Sise ME, Bloom AK, Wisocky J, et al. Treatment of hepatitis C virus-associated mixed cryoglobulinemia with direct-acting antiviral agents. *Hepatology. 2016;63:408–417.

19. European Association for the Study of the Liver. EASL recommendations on treatment of hepatitis C 2016. *J Hepatol. 2017;66:153–194.

20. AASLD/IDSA HCV Guidance Panel. Hepatitis C guidance: AASLD-IDSA recommendations for testing, managing, and treating adults infected with hepatitis C virus. *Hepatology. 2015;62:932–954.

21. Sollima S, Milazzo L, Peri AM, et al. Persistent mixed cryoglobulinaemia vasculitis despite hepatitis C virus eradication after interferon-free antiviral therapy. *Rheumatology (Oxford). 2016;55:2084–2085.

22. Ghosn M, Palmer MB, Najem CE, et al. New-onset hepatitis C virus-associated glomerulonephritis following sustained virologic response with direct-acting antiviral therapy. *Clin Nephrol. 2017;87:261–266.

23. Chowdhury R, Tsen A. Recurrent mixed cryoglobulinemia despite sustained virologic response to treatment: a case report. *Am J Kidney Dis. 2017;70:301–304.

24. Terrier B, Joly F, Vazquez T, et al. Expansion of functionally anergic CD21-low marginal zone-like B cell clones in hepatitis C virus infection-related autoimmunity. *J Immunol. 2011;187:6550–6563.

25. Comarmond C, Garrido M, Pol S, et al. Direct-acting antiviral therapy restores immune tolerance to patients with hepatitis C virus-induced cryoglobulinemia vasculitis. *Gastroenterology. 2017;152:2052–2062.

26. Cornella SL, Stine JG, Kelly V, et al. Persistence of mixed cryoglobulinemia despite cure of hepatitis C with new oral antiviral therapy including direct-acting antiviral sofosbuvir: a case series. *Postgrad Med. 2015;127:413–417.

27. Del Padre M, Todi L, Mitrevski M, et al. Reversion of anergy signatures in clonal CD21low B cells of mixed cryoglobulinemia after clearance of HCV viremia. *Blood. 2017;130:35–38.

28. Reyes-Aviles E, Kostadima L, Rusterholtz A, et al. Presence of rheumatoid factor during chronic HCV infection is associated with expansion of mature activated memory B-cells that are hypo-responsive to B-cell receptor stimulation and persist during the early stage of IFN-free therapy. *PLoS One. 2015;10:e0144629.

29. Schiavinato A, Zanetto A, Pantano G, et al. Polyclonal and monoclonal B-lymphocytes response in HCV patients treated during the early stage of IFN-free therapy. *Blood. 2015;126:1176–1182.

30. Aivado MS, Diament JH, Kaplan LM, et al. Polyclonal and monoclonal B-lymphocytes response in HCV patients treated during the early stage of IFN-free therapy. *Blood. 2015;126:1176–1182.

31. Riche J, Ounnian A, Girard M, et al. High prevalence of hepatitis C virus RNA in the supernatant of the cryoprecipitate of patients with essential and secondary type II mixed cryoglobulinemia. *J Hepatol. 1994;21:58–63.

32. Dammacco F, Sansonno D, Cornacchini V, et al. Hepatitis C virus infection and mixed cryoglobulinemia: a striking association. *Int J Clin Lab Res. 1993;23:45–49.