Digestive Diseases and Sciences
https://doi.org/10.1007/s10620-025-09033-8

ORIGINAL ARTICLE

Long Term Follow-Up of Patients with Cryoglobulinemia After Successful Treatment of Chronic C Virus Hepatitis

Bianka Zsuzsa Elthes[1,2] · Zsuzsanna Vitalis[1] · Maria Papp[1] · Tamas Tornai[3,4] · David Tornai[1] · Istvan Tornai[1,2]

Received: 15 January 2025 / Accepted: 30 March 2025
© The Author(s) 2025

Abstract

Background Mixed cryoglobulinemia is one of the most important extrahepatic manifestations of chronic hepatitis C infection.

Aims and Methods We screened 111 HCV-infected patients and identified 40 with cryoglobulinemia, who later achieved sustained virologic response (SVR). We prospectively followed them regarding laboratory findings and clinical symptoms for a median [IQR] of 5 [3–10] years.

Results Prior to antiviral treatment, the median serum cryoglobulin level was 297 (IQR: 61–1144) mg/L. In 25 patients type II, while in 15 type III cryoglobulinemia were found with significant difference in cryoglobulin levels (669 [297–2713] vs. 57 [33–123], respectively) ($p < 0.001$). Only 23 patients had clinical symptoms at the diagnosis, of whom 21 had cryoglobulinemic vasculitis and 2 non-Hodgkin's lymphoma (NHL), and 17 patients were asymptomatic. Cryoglobulin levels were monitored yearly after SVR. Median times to cryoglobulin disappearance were significantly different between type II and type III disease forms (36 vs. 12 months, pLog-Rank: 0.002). Improvement or complete cessation of complaints were parallel to the cryoglobulin disappearance. Vasculitis, in most cases (n = 16) and one NHL were cured spontaneously during follow-up observation. However, some patients required specific treatment, such as immunosuppression [n = 5] for vasculitis and combined chemotherapy [n = 1] for aggressive NHL. Relapses of cryoglobulinemia and related symptoms were detected in 2 patients. Importantly, polyneuropathy did not show improvement by any means.

Conclusions Our results support that the monitoring of cryoglobulins is important even after SVR, especially in case of type II forms. Long-term complications such as severe vasculitis or NHL may still occur.

Keywords Cryoglobulinemia · HCV · Vasculitis · NHL · Immunosuppressive treatment

Abbreviations

CG	Cryoglobulin
DAA	Direct acting antiviral drugs
DLBCL	Diffuse large B cell lymphoma
F	Fibrosis
HCV	Hepatitis C virus
IFN	Interferon
IgG	Immunoglobulin G
IgM	Immunoglobulin M
NHL	Non-Hodgkin's lymphoma
RF	Rheumatoid factor
RNA	Ribonucleic acid
SVR	Sustained virologic response
WHO	World Health Organization

✉ Bianka Zsuzsa Elthes
elthes.zsuzsa@med.unideb.hu

1 Division of Gastroenterology, Department of Internal Medicine, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

2 Faculty of Dentistry, Doctoral School of Dental Sciences, University of Debrecen, Debrecen, Hungary

3 Institute for Pancreatic Disorders, Semmelweis University, Budapest, Hungary

4 Department of Gastroenterology, Gent University Hospital, Ghent, Belgium

Introduction

According to WHO estimates, hepatitis C virus (HCV) infection affects approximately 2–3% of the world's population, with an expected number of 350.000 deaths every year [1–3]. Besides its hepatotoxic effects, chronic HCV infection can trigger a wide range of extrahepatic manifestations, which can increase mortality [4]. One of the most common

Published online: 10 April 2025

extrahepatic manifestations is mixed cryoglobulinemia. This phenomenon may be present in up to two-thirds of the patients with chronic HCV infection [5] and is characterized by the presence of circulating cryoglobulins (CG). These abnormal proteins precipitate at low temperature (< 37 °C) and dissolve when the temperature rises. Cryoglobulins are produced by chronically stimulated B lymphocytes. Hepatitis C virus infection leads to continuous stimulation of the immune system. As a result, B cells produce polyclonal immunoglobulin G (IgG), and monoclonal or polyclonal immunoglobulin M (IgM), with rheumatoid factor (RF) activity. Chronic HCV infection is known to be associated with type II or type III cryoglobulinemia. While type II cryoglobulinemia is characterized by the presence of monoclonal IgM and polyclonal IgG, in type III cryoglobulinemia polyclonal IgM and IgG antibodies are present [6].

Cryoglobulinemia can cause a wide range of clinical symptoms. The main clinical manifestations can be on the one hand cryoglobulinemic vasculitis (CV), such as purpura or necrotizing ulcers, glomerulonephritis, arthralgia, peripheral polyneuropathy and some other clinical presentations. In most severe cases cryoglobulinemia leads to the development of non-Hodgkin's lymphoma (NHL) [7–9]. The presence of CGs is associated with a 35-fold higher risk for developing B-cell NHL as compared to normal population [10]. It is widely believed that B cell stimulation progressively becomes less dependent on HCV infection. The development of monoclonal immunoglobulins, specifically in type II cryoglobulinemia, could mark an important milestone, with NHL being the most serious complication associated with HCV infection and persistent B cell stimulation [10–12]. Over a decade ago, replacement of interferon by direct-acting antiviral agents (DAA) was a major advancement in HCV treatment, achieving over 95% sustained virologic response (SVR). Despite achieving SVR and favorable clinical outcomes with DAA therapy, a notable proportion of patients with hepatitis C virus-associated cryoglobulinemic vasculitis (HCV-CV) remain positive for cryoglobulins, and relapses of vasculitis have been reported. Studies indicate that more than 10% of HCV-CV patients may experience vasculitis relapses even after DAA-induced SVR, with relapses often being moderate to severe [13–15]. These findings underscore the need for ongoing monitoring of HCV-CV patients after achieving SVR, to promptly identify and manage potential relapses in laboratory findings and/or clinical symptoms.

In this study, we prospectively followed patients with HCV infection, who signed the informed consent prior to antiviral treatment and later achieved SVR at our University Clinic. Before HCV treatment we assessed the presence of cryoglobulinemia and related symptoms. After achieving SVR, we performed long-term evaluation of laboratory results and clinical outcomes to determine the resolution rate of cryoglobulinemia and its time frame. For patients with persistent cryoglobulinemia, we assessed symptom progression and the potential need for immunosuppressive therapy.

Patients and Methods

Between 2012 and 2022, out of 317 patients with newly diagnosed HCV infection 111 signed the informed consent for a thorough investigation of cryoglobulinemia and a possible long-term prospective follow-up in case of successful antiviral treatment at the Department of Gastroenterology, University of Debrecen. Either interferon-based regime containing primary generation protease inhibitor telaprevir (n = 9 patients) or a combination of DAAs (n = 102 patients) were administered. Direct acting antiviral drugs containing regimes included sofosbuvir plus ledipasvir or elbasvir plus grazoprevir or ombitasvir plus paritaprevir/ritonavir plus dasabuvir. According to standard criteria, SVR was considered as undetectable HCV RNA level at 12 weeks after the end of treatment. All patients received routine laboratory assessments (blood count, biochemistry, coagulation), which included cryoglobulinemia testing as well. Upon detection of cryoglobulinemia, from the same sample we measured cryoglobulin serum concentration, rheumatoid factor (RF) IgM activity, and C4 factor levels [16, 17]. Cryoglobulins were classified as type II when monoclonal IgM was detected with polyclonal IgG, and as type III if polyclonal IgG and IgM were found. Detailed assessments were conducted for patients with cryoglobulinemia by collecting complaints and clinical symptoms, noting the presence of different manifestations of CV or NHL. Diagnosis of peripheral neuropathy was based on neurological examination and electromyography. Raynaud's phenomenon, Sjögren's syndrome and myopathy were diagnosed by rheumatological examination. Fibrosis was evaluated through transient elastography (Fibroscan, Echosense) or shear wave elastography (Aixplorer Supersonic Imagine). Using the METAVIR scale, stages F1-2 were identified if liver stiffness was below 9.6 kPa, and F3-4 if it measured 9.6 kPa or more.

Follow-Up After Treatment

Cryoglobulins, RF IgM activity and C4 levels were determined yearly after SVR, until CGs were undetectable. However, 39 patients were followed-up further due to the advanced fibrotic stage and to perform HCC surveillance while 1 patient was lost to follow-up one year after SVR. In patients with persistent cryoglobulinemia and severe clinical symptoms (vasculitis or NHL) we administered disease specific treatment such as: immunosuppression, including rituximab or prednisolone plus azathioprine or chemotherapy.

Statistical Analysis

Continuous variables were summarized as medians and interquartile range (IQR; 25-75th percentiles). Continuous variables were compared with Mann–Whitney U-test, Wilcoxon matched-pairs signed rank test. Categorical variables were compared with Fisher's exact test.

Kaplan–Meier curve was used to demonstrate the long-term follow-up of the presence of cryoglobulins in patients. For the comparison of survival curves *Log-rank* (Mantel-Cox) test was used. For statistical analysis and graphical presentation Graph Pad Prism 6 (San Diego, California, United States) was used. A two-sided probability value of < 0.05 was considered to be statistically significant.

Table 1 Demographic data and baseline laboratory results of patients who tested cryoglobulin-positive before treatment for chronic hepatitis C virus infection

	Patients with cryoglobulinemia
Number of patients: n (%)	40 (100%)
Female/Male (n)	30/10
Age (median; IQR) year*	59 (53–63)
Stage of fibrosis: n (%)	
F1–2 stage: < 9.6 kPa	5 (12.5%)
F3–4 stage: ≥ 9.6 kPa	35 (87.5%)
Type of cryoglobulin: n (%)	
Type II:	25 (62.5%)
Type III:	15 (37.5%)
Total cryoglobulin protein (mg/L)	297 (61–1144)
Rheumatoid factor IgM activity (IU/mL)	152.05 (10.83–1059)
Complement factor 4 (g/L)	0.07 (0.04–0.11)

kPa: kiloPascal

*Data are given as median and interquartile range

Results

Patient's Characteristics and Laboratory Results Before Antiviral Treatment

Out of 111 patients, cryoglobulins were positive in 40 (36.6%), while negative in 71 (63.4%). Demographic data, such as age and gender were similar in both groups. No significant difference was detected in various laboratory data, namely blood count, liver enzymes and liver function tests neither in other biochemistry or liver stiffness values (*data not shown*).

Cryoglobulin positive patients were evaluated further in the present study. As shown in Table 1, the median age of these patients was 59 [IQR, 53–63] years and advanced fibrosis (F3-4 stage) was detected in 87.5%. They were more commonly females (75%). Type II cryoglobulinemia was present in 25 patients (62.5%) while 15 patients (37.5%) had type III cryoglobulinemia. In the majority of patients (73%) C4 levels were below the lower limit of normal (LLN, 0.1 g/L), as expected. We compared the total CG levels, the related RF IgM activity and the C4 levels in patients with type II and type III cryoglobulins (Table 2). We found significant differences in the median CG levels (669 [297–2713] mg/L vs. 57 [33–123] mg/L) and RF IgM activity (870 (326–1617) IU/ml vs. 10 (10–17) IU/ml), respectively ($p < 0.001$). In patients with type III cryoglobulins C4 levels were significantly higher than those with type II cryoglobulins: 0.08 (0.06–0.12) vs. 0.06 (0.03–0.08) g/L ($p = 0.03$).

At baseline, only 23 out of 40 patients (57.5%) had clinical symptoms related to cryoglobulinemia. Nineteen patients (82.6%) out of these 23 with clinical symptoms had type II cryoglobulinemia (Table 3). However, in patients without clinical symptoms, type III cryoglobulinemia was the dominant one, and found in 11 out of 17 cases (64.7%). Furthermore, we compared the total cryoprotein [657.3 (230–1914) vs. 71.8 (40.9–273) mg/L; $p < 0.01$], RF IgM activity [943 (150.3–2244) vs. 10.2 (10–23.8) UI/mL; $p < 0.001$] and C4 levels [0.05 (0.03–0.08) vs. 0.10 (0.06–0.12) g/L; $p < 0.01$] in patients with or without clinical symptoms and found

Table 2 Levels of total cryoglobulin protein, rheumatoid factor IgM activity and complement factor 4 in all 40 patients with type II or type III cryoglobulinemia before antiviral treatment

	Type II CG (n = 25)	Type III CG (n = 15)	p value*
Total cryoglobulin protein (mg/L)	669 (297–2713)	57 (33–123)	< 0.001
Rheumatoid factor IgM activity (IU/mL)	870 (326–1617)	10 (10–17)	< 0.001
Complement factor 4 (g/L)	0.06 (0.03–0.08)	0.08 (0.06–0.12)	0.03

Data are given as median and interquartile range (25–75%)

CG Cryoglobulin

*p-Value was calculated by Mann–Whitney U-test

Table 3 Laboratory and some demographic data of cryoglobulin positive patients at baseline with or without clinical manifestations

	Patients with symptomatic cryoglobulinemia	Patients with asymptomatic cryoglobulinemia	p value
Number of patients: n (%)	23 (57.5%)	17 (42.5%)	NA
Age (median; IQR) year*	59 (53–63)	57 (53.5–68.5)	NS
Fibroscan/Elastography (kPa) median (IQR)	15 (10–23.1)	17.7 (11–39.5)	NS
Cryoglobulinemic vasculitis NHL	21 2	0 0	NA
Type of cryoglobulin: n (%)			
Type II:	19 (82.6%)	6 (35.3%)	0.003
Type III:	4 (17.4%)	11 (64.7%)	
Total cryoglobulin protein (mg/L)	657.3 (230–1914)	71.81 (40.87–273)	< 0.01
Rheumatoid factor IgM activity (IU/mL)	943 (150.3–2244)	10.15 (10–23.8)	< 0.001
Complement factor 4 (g/L)	0.05 (0.03–0.08)	0.10 (0.06–0.12)	< 0.01

*Data are given as median and interquartile range

NA not applicable, *NS* nonsignificant

p-Value was calculated by Fisher's exact test or Mann–Whitney U-test

Table 4 Clinical manifestations at baseline and at one year after sustained virologic response

	Baseline	One year after SVR
Patients with clinical manifestations (n)	23	13
Cryoglobulinemic vasculitis	21	12
Skin purpuras	11	7
Glomerulonephritis	9	3
Arthralgia	4	2
Polyneuropathy	4	4
Raynaud's phenomenon	1	1
Sjögren's syndrome	1	1
Myopathy	1	0
NHL	2	1

Combination of more clinical manifestations was seen in 5 patients

NHL Non-Hodgkin's lymphoma, *SVR* Sustained virologic response

all of them to be significantly different between these two groups (Table 3).

Clinical Symptoms Before Antiviral Treatment

Cryoglobulinemic vasculitis manifesting as skin purpura, skin ulcers, glomerulonephritis, arthralgia, peripheral sensory polyneuropathy, myopathy, Raynaud's phenomenon as well as Sjögren's syndrome could be observed in 21 patients. In 5 patients more than one of these manifestations were present, as shown in Table 4. Non-Hodgkin's lymphoma, as the most severe consequence of cryoglobulinemia, was diagnosed in two patients, and both had type II cryoglobulins. One patient had aggressive diffuse large B cell lymphoma (DLBCL), and the other patient had indolent marginal zone lymphoma. The patient with DLBCL received antiviral therapy only after achieving remission with chemotherapy (R-CHOP regimen). The patient with marginal zone lymphoma has received antiviral drugs as primary therapy, which cured the NHL as well. No chemotherapy was needed later, and he has been in complete remission so far.

As previously described, we have found 17 patients with asymptomatic cryoglobulinemia. When comparing their age, liver enzyme levels and fibrosis level, there was no significant difference in the results between the symptomatic and asymptomatic cryoglobulinemic groups. Only the levels of cryoglobulins showed significant differences between these two groups (Table 3).

Laboratory Outcomes After the Successful Antiviral Treatment

The enrolled patients were followed up between 2012 and 2024. The median (IQR) follow-up time was 5 (3–10) years. In 23 patients, 10 with type II and 13 with type III cryoglobulinemia, cryoproteins became undetectable within the first year after achieving SVR. Their initial cryoglobulin level was only 98 (42–326) mg/L, in contrast to 1100 (373–3121) mg/L in the 17 patients who still had detectable cryoglobulin after 1 year. As shown in Fig. 1 this high level decreased to 128 (39–1208) mg/L after the antiviral treatment ($p < 0.001$). RF IgM activity showed a similar pattern *(data not shown)*. In the group of patients who remained

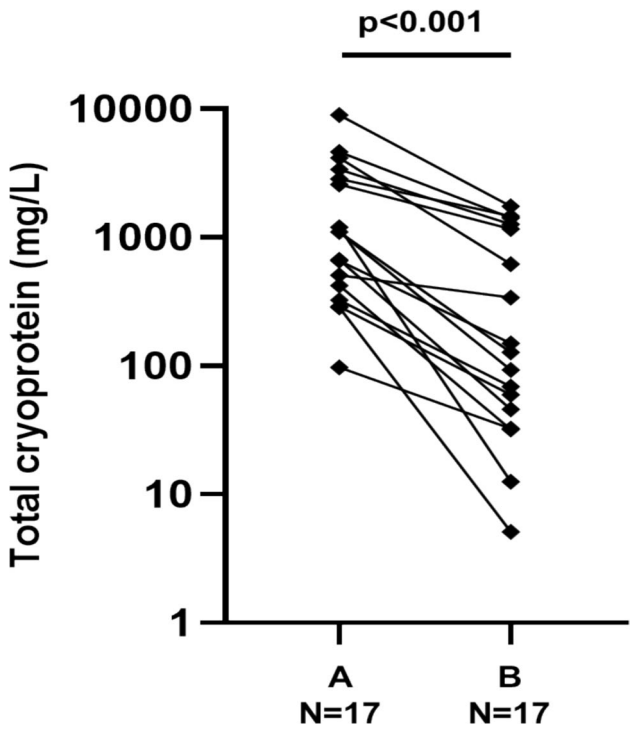

Fig. 1 Total cryoglobulin levels in 17 patients at baseline (**A**) and after one year follow-up (**B**) in whom cryoglobulins remained positive after sustained virologic response. p-value was calculated by Wilcoxon matched-pairs signed rank test

positive for cryoglobulins after one year of achieving SVR, 15 had type II cryoglobulinemia, and 2 had type III.

The disappearance rate of cryoglobulins is demonstrated in Fig. 2. Interestingly, every patient with type III cryoglobulinemia became cryoglobulin negative in four years. While

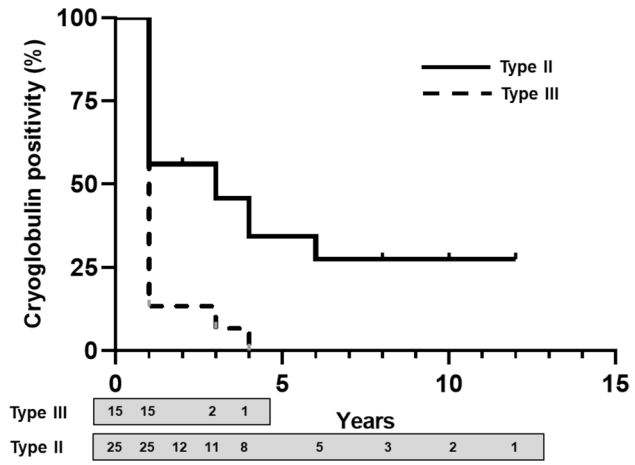

Fig. 2 Follow up of the presence of type II and type III cryoglobulins in patients after successful antiviral therapy. Kaplan–Meier curves are shown. p-value was calculated by Log-rank (Mantel-Cox) test and proved to be < 0.01

in those patients with type II cryoglobulinemia, the disappearance rate was much slower. Median time to disappearance of cryoglobulins was significantly different between type III and type II disease forms (12 vs. 36 months, p*Log-Rank*: 0.002). The C4 level increased after antiviral treatment as shown in Fig. 3. The median pre-treatment level was 0.07 (0.04–0.11) mg/L. In those who had detectable cryoglobulin at the end of the first year after antiviral therapy, C4 level remained at a similar level, however, in those achieving cryoprotein negative status 0.16 (0.12–0.22) mg/L was detected ($p < 0.001$). Only 4 patients' C4 levels did not reach the normal range and remained below 0.1 mg/L.

In the group of patients with asymptomatic cryoglobulinemia (n = 17), within the first year after SVR 13 (76%) patients became cryoglobulin negative (Type II 4 and Type III 9 patients). In this group we did not observe any cryoglobulinemic relapse or the appearance of any autoimmune diseases or cancers during the follow-up period.

Follow-Up of Clinical Manifestations

The cryoprotein level was significantly higher in those 23 patients who had clinical manifestations at baseline as shown in Table 3. Within one year follow-up we observed the disappearance of cryoproteins as well as the concurrent clinical manifestations in 10 patients (Table 4). Among them, 9 patients had vasculitis (with different manifestations) and one patient had marginal zone NHL. The cryoprotein level of these 10 patients at baseline had a trend of being lower

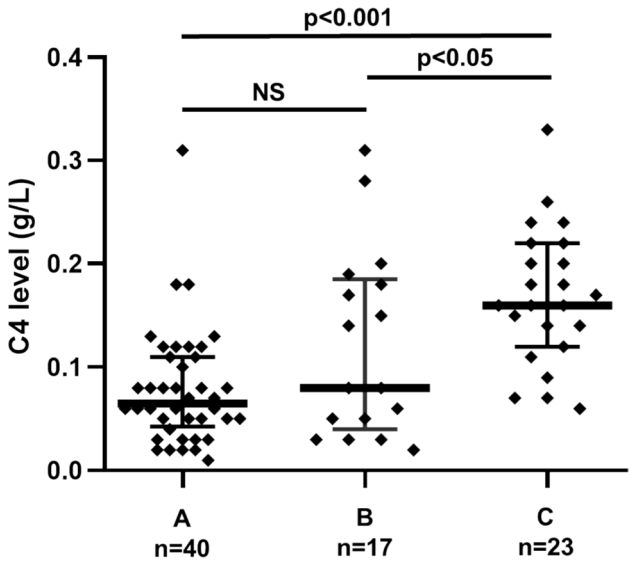

Fig. 3 Complement factor 4 levels in all 40 patients at baseline before treatment (**A**) and at one year after antiviral treatment separately, according to cryoglobulin positive (**B**) or negative (**C**) status. Lines denote median values, error bars represent 25th to 75th percentiles. p-value was calculated by Mann–Whitney U-test

than that of 13 patients who still had clinical symptoms after one year [315.4 (87.3–1347) vs. 1100 (415–2713) mg/L], but the difference was only close to being statistically significant ($p = 0.08$). As shown in Table 4. at one year after SVR, we still observed vasculitis in 12 patients, and aggressive NHL, i.e. DLBCL, in 1. The symptoms of the vasculitis were mild or moderate in 7 patients (3 glomerulonephritides, 2 arthralgias and 2 skin purpuras) and parallel to the disappearance of cryoglobulins they were spontaneously cured within a few years. However, in 5 patients skin purpuras, combined with mild to moderate polyneuropathy or Raynaud's phenomenon, were so severe that immunosuppressive treatment became necessary. In 4 patients, rituximab was the choice of treatment and in a patient with necrotizing skin vasculitis, azathioprine and prednisolone were administered. The severe vasculitis was cured in all 5 patients. In one of them we observed a temporary disappearance of cryoproteins, which was followed by a relapse of vasculitis and cryoproteins after a short period of time. Later they spontaneously and permanently disappeared. However, neither polyneuropathy nor Raynaud's phenomenon have shown any improvement, even after up to ten-year follow-up, independently from the cryoprotein status. The patient with Sjögren's syndrome has not been treated with immunosuppressive treatment due to the mild symptoms and low impact on quality of life. In the patient with DLBCL, antiviral treatment was administered only after achieving full remission with R-CHOP therapy. After this cryoproteins disappeared for a short period of time. However, when cryoglobulins reappeared NHL relapsed, as well. In this case, despite the repeated R-CHOP therapy, remission could not be achieved for a second time and the patient died 2 years after SVR. During the observation period another patient died 4 years after SVR due to cardiovascular disease. This patient also was cryoprotein positive at the time of death.

Discussion

In our prospective chronic HCV patient cohort, we aimed to evaluate changes in the serum levels of cryoproteins and in cryoprotein associated disorders, like cryoglobulinemic vasculitis and/or lymphoma, after successful antiviral therapy. HCV eradication and subsequent disappearance of cryoglobulins in patients treated with IFN and ribavirin [18, 19] or pegylated IFN and ribavirin combination [20, 21] was previously demonstrated. Recently the efficacy of DAA therapy on remission of cryoglobulins and its complications has also been documented, however there are only a few studies with long-term follow-up [22–26]. In the majority of our patients DAA therapy was used. Although we investigated a small patient cohort, but with an exceptionally long follow-up period (more than 12 years) that is the longest evaluation period to our knowledge following SVR in patients with mixed cryoglobulinemia. In our study serum levels of cryoglobulins and type of immunoglobulins were detected. Thus, type II and type III forms could be precisely identified. Type II cryoglobulins with monoclonal IgM content were present in most patients (25 type II vs. 15 type III). A novel finding of the present study is a striking quantitative difference, i.e. more than 10 times higher amount of total cryoprotein content, between type II and type III cryoglobulins. Similar differences in RF IgM activity were detected according to type of cryoglobulinemia as well. This demonstrates that in these cases, B cell stimulation is probably much stronger [10], which might explain why the time needed until the full disappearance of type II cryoproteins was also much longer following the eradication of HCV infection as compared to type III. More than 50% of our patients became cryoglobulin negative within one year after achieving SVR. The initial cryoglobulin levels in these 23 patients, however, were 10 times lower as compared to those, who remained cryoprotein positive at that time point. The vast majority of cases in the positive group had type II cryoproteins (15 out of 17), but even their total cryoprotein levels have already decreased significantly by cc. 90% within the first year. Cryoglobulins disappeared within 4 years after SVR in all type III patients, whereas we still have positive patients with type II cryoglobulins even after 12 years of follow up. C4 results are in line with previous observations, i.e. in the presence of cryoglobulins C4 is significantly lower than the lower limit of normal range and C4 normalizes after SVR parallel to the disappearance of cryoglobulins [27].

Due to the prospective design of this study, we have detected 17 patients with asymptomatic cryoglobulinemia. During the follow-up period, all of them remained asymptomatic. The significantly lower level of cryoglobulins compared to symptomatic patients could at least partially explain the absence of clinical symptoms in this group. Symptomatic cryoglobulinemia (benign vasculitis or NHL) was associated with a 9 times higher total protein content as compared to asymptomatic cryoglobulinemia. Furthermore, type II cryoglobulins were present in more than 80% of the symptomatic patients, whereas in asymptomatic patients type III cryoglobulin was the dominant one.

As expected, in our patients, the typical manifestations of cryoglobulinemic vasculitis were skin purpuras, skin ulcers, glomerulonephritis, arthralgias, polyneuropathy and some rare types, like Raynaud's phenomenon, myopathy, or Sjögren's syndrome. Another important observation was the presence of more than one manifestation of cryoglobulinemic vasculitis at the same time in several patients. We also observed immediate improvement of vasculitis after achieving SVR in most cases. In 43% of these patients (10 out of 23), vasculitis disappeared within one year after eradication of HCV. However, in five patients skin purpuras were

so severe that immunosuppressive therapy became necessary. In three patients, sensory polyneuropathy and in one Raynaud's phenomenon were also present. Fortunately, in their cases, with additional therapy, vasculitis could be effectively cured and cryoglobulins also disappeared in 2 of them. But sensory polyneuropathy or Raynaud's phenomenon did not show significant improvement during the observation period, despite the treatment with rituximab.

The most severe consequence of HCV infection and mixed cryoglobulinemia is the NHL. In one of our patients treated for DLBCL, we could see a temporary disappearance of cryoglobulins after achieving SVR. But when type II cryoglobulins reappeared, NHL also relapsed, which proved to be fatal in this case. Fortunately, in line with the current treatment guidelines, the patient with indolent marginal zone NHL could be permanently cured only with HCV eradication. During the follow-up, we did not detect cryoglobulin relapses although he also had type II cryoglobulins.

In two long-term follow-up trials, relapse or "de-novo" appearance of cryoglobulins have been observed among patients who were successfully treated with chronic HCV infection, using DAA therapy. Fayed et al. [15] could observe 12.6% vasculitis relapses in 913 patients. Importantly, half of patients with relapse required aggressive treatment, such as plasma exchange or cyclophosphamide. They concluded that relapse, even after successful DAA therapy, seems to be a big challenge. In a study from Taiwan, Chang et al. [28] determined the number of different cryoglobulin components (IgM, IgA, IgG) in their patients, but absolute amount or type of cryoglobulinemia were not reported. They similarly detected the recurrence of cryoproteins in some of their patients after obtaining cryoglobulin negative status. Surprisingly, they observed appearance of cryoglobulinemia in 9% of the originally cryoprotein negative patients, despite achieving SVR during the 4-year follow-up. It was suggested that relapse and/or "de novo" appearance could be associated either with infection or malignancy, which was also observed in our patient with DLBCL. Although, we did not reassess cryoglobulin status of our initially cryoglobulin negative patients, none of them developed any manifestations of cryoglobulinemic vasculitis during follow-up. Fluctuation of cryoprotein levels either relapse or "de novo" appearance seems to indeed suggest an independent B cell stimulation induced by the earlier HCV infection. Interestingly, only less than 2% of cryoglobulinemic patients had vasculitis in that large Asian cohort. Certainly, this is not typical for the Caucasian population. In a recent Italian publication, the PITER study by Kondili et al. [29], the authors followed a large cohort of patients with cryoglobulinemia after DAA treatment, and also observed clinical relapse in 13% of their patients after SVR. Infection as well as malignancy seemed to be two important risk factors for relapse, as was observed in our patient with aggressive NHL.

The limitation of our study is the small number of enrolled patients. However, we have interesting new details like precise typing and absolute amount of cryoproteins associated with clinical manifestations and the longest follow up period (12 years) so far after achieving SVR. We could also confirm that similar or better results can be achieved by using DAA as previously reported with IFN therapy.

In conclusion, our findings demonstrate that type II cryoglobulins are predominant in patients with mixed cryoglobulinemia and are primarily associated with clinical symptoms. After achieving SVR, patients with type III cryoglobulins show a more rapid resolution of both clinical manifestations and cryoglobulins. However, vasculitis can persist at a severity that requires immunosuppressive treatment even post-SVR. These results underscore the importance of long-term monitoring for cryoglobulinemic patients, given the potential risk of relapse of vasculitis or developing NHL, regardless of SVR status.

Author Contributions Bianka Zsuzsa Elthes: data collection, data analysis, writing the manuscript. Zsuzsanna Vitalis: consultation of the manuscript, patient treatment. Maria Papp: study design, patient treatment, consultation of the manuscript. Tamas Tornai: patient treatment, consultation of the manuscript. David Tornai: consultation of the manuscript, data analysis. Istvan Tornai: study design, data analysis, patient treatment, writing the manuscript.

Funding Open access funding provided by University of Debrecen.

Data Availability No datasets were generated or analysed during the current study.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The study design was approved by the Regional and Institutional Research Ethics Committee (RKEB/IKEB 5306-9/2011). All patients gave their consent to this prospective trial and all investigations were in line with the declaration of Helsinki.

Open Access This article is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License, which permits any non-commercial use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by-nc/4.0/.

References

1. Mohd Hanafiah K, Groeger J, Flaxman AD, Wiersma ST. Global epidemiology of hepatitis C virus infection: new estimates of age-specific antibody to HCV seroprevalence. *Hepatology.* 2013;57:1333–1342.
2. 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.
3. Denniston MM, Jiles RB, Drobeniuc J et al. Chronic hepatitis C virus infection in the United States, National Health and Nutrition Examination Survey 2003 to 2010. *Ann Intern Med.* 2014;160:293–300.
4. Kuna L, Jakab J, Smolic R et al. HCV Extrahepatic Manifestations. *J Clin Transl Hepatol.* 2019;7:172–182.
5. Cacoub P, Vieira M. Saadoun D Cryoglobulinemia-One Name for Two Diseases. *N Engl J Med.* 2024;391:1426–1439.
6. Damoiseaux J, Tervaert JWC. Diagnostics and Treatment of Cryoglobulinemia: It Takes Two to Tango. *Clin Rev Allergy Immunol.* 2014;47:299–310.
7. Cacoub P, Saadoun D. Extrahepatic manifestations of chronic HCV infection. *N Engl J Med.* 2021;384:1038–1052.
8. Monti G, Pioltelli P, Saccardo F et al. Incidence and Characteristics of Non-Hodgkin Lymphomas in a Multicenter Case File of Patients With Hepatitis C Virus-Related Symptomatic Mixed Cryoglobulinemias. *Arch Intern Med.* 2005;165:101–105.
9. Dal Maso L, Franceschi S. Hepatitis C virus and risk of lymphoma and other lymphoid neoplasms: a meta-analysis of epidemiologic studies. *Cancer Epidemiol Biomarkers Prev.* 2006;15:2078–2085.
10. Cheng YT, Cheng JS, Lin CH, Chen TH, Lee KC, Chang ML. Rheumatoid factor and immunoglobulin M mark hepatitis C-associated mixed cryoglobulinemia: an 8-year prospective study. *Clin Microbiol Infect.* 2020;26:366–372.
11. 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.e2.
12. Visentini M, Del Padre M, Colantuono S et al. Long-lasting persistence of large B-cell clones in hepatitis C virus-cured patients with complete response of mixed cryoglobulinaemia vasculitis. *Liver Int.* 2019;39:628–632.
13. Cacoub P, Si Ahmed SN, Ferfar Y et al. Long-term Efficacy of Interferon-Free Antiviral Treatment Regimens in Patients With Hepatitis C Virus-Associated Cryoglobulinemia Vasculitis. *Clin Gastroenterol Hepatol.* 2019;17:518–526.
14. Bonacci M, Lens S, Mariño Z et al. Long-Term Outcomes of Patients With HCV-Associated Cryoglobulinemic Vasculitis After Virologic Cure. *Gastroenterology.* 2018;155:311-315.e6.
15. Fayed A, Hegazy MT, Biard L et al. Relapse of Hepatitis C Virus Cryoglobulinemic Vasculitis After Sustained Viral Response After Interferon-Free Direct-Acting Antivirals. *Am J Gastroenterol.* 2022;117:627–636.
16. Musset L, Diemert MC, Taibi F et al. Characterisation of cryoglobulins by immunoblotting. *Clin Chem.* 1992;38:798–802.
17. Stoyanov A, Toong C, Kong Y, Chen R, Urriola N. Serum protein electrophoresis and rheumatoid factor analysis is an effective screening strategy for cryoglobulinaemia. *Pathology* 2023;55:391–396.
18. Mazzaro C, Zorat F, Comar C et al. Interferon plus ribavirin in patients with hepatitis C virus positive mixed cryoglobulinemia resistant to interferon. *J Rheumatol* 2003;30:1775–1781.
19. Cacoub P, Lidove O, Maisonobe T et al. Interferon-α and ribavirin treatment in patients with hepatitis C virus-related systemic vasculitis. *Arthritis Rheum.* 2002;46:3317–3326.
20. 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. *Clinical and Experimental Rheumatology.* 2011;29:933–941.
21. Gragnani L, Fognani E, Piluso A, MaSVE Study Group et al. Long-term effect of HCV eradication in patients with mixed cryoglobulinemia: a prospective, controlled, open-label, cohort study. *Hepatology.* 2015;61:1145–1153.
22. Mazzaro C, Dal Maso L, Mauro E et al. Hepatitis C virus-related cryoglobulinemic vasculitis: A review of the role of the new direct antiviral agents (DAAs) therapy. *Autoimmun Rev.* 2020;19:102589.
23. 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.
24. Gragnani L, Visentini M, Fognani E et al. Prospective study of guideline-tailored therapy with direct-acting antivirals for hepatitis C virus-associated mixed cryoglobulinemia. *Hepatology.* 2016;64:1473–1482.
25. Bonacci M, Lens S, Londoño 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.e1.
26. Comarmond C, Cacoub P, Saadoun D. Treatment of chronic hepatitis C-associated cryoglobulinemia vasculitis at the era of direct-acting antivirals. *Therap Adv Gastroenterol.* 2020;13:1756284820942617.
27. Chang ML, Hu JH, Chen WT, Lin MS, Kuo CJ, Chen SC, Chien RN. Interactive Impacts from Hepatitis C Virus Infection and Mixed Cryoglobulinemia on Complement Levels. *Dig Dis Sci.* 2021;66:2407–2416. https://doi.org/10.1007/s10620-020-06507-9.
28. Chang ML, Cheng JS, Chuang YH et al. Evolution of Cryoglobulinemia in Direct-Acting Antiviral-Treated Asian Hepatitis C Patients With Sustained Virological Responses: A 4-Year Prospective Cohort Study. *Front Immunol.* 2022;13:823160.
29. Kondili LA, Monti M, Quaranta MG et al. A prospective study of direct-acting antiviral effectiveness and relapse risk in HCV cryoglobulinemic vasculitis by the Italian PITER cohort. *Hepatology.* 2022;76:220–232.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.