



HAL
open science

Cryoglobulinemia after the era of chronic hepatitis C infection

Gonçalo Boleto, Pascale Ghillani-Dalbin, Lucile Musset, Lucie Biard,
Guillaume Mulier, Patrice Cacoub, David Saadoun

► **To cite this version:**

Gonçalo Boleto, Pascale Ghillani-Dalbin, Lucile Musset, Lucie Biard, Guillaume Mulier, et al.. Cryoglobulinemia after the era of chronic hepatitis C infection. *Seminars in Arthritis and Rheumatism*, 2020, 50, pp.695 - 700. 10.1016/j.semarthrit.2020.05.004 . hal-03490783

HAL Id: hal-03490783

<https://hal.science/hal-03490783>

Submitted on 9 Jun 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

Cryoglobulinemia after the era of chronic hepatitis C infection

Gonçalo Boleto, MD^{a,b,c,d}; Pascale Ghillani-Dalbin PharmD, PhD^e; Lucile Musset PharmD, PhD^e; Lucie Biard MD, PhD^f; Guillaume Mulier MD^f; Patrice Cacoub MD, PhD^{a,b,c,d*}; David Saadoun MD, PhD^{a,b,c,d*}

^aSorbonne Université, UPMC Univ Paris 06, UMR 7211, Inflammation-Immunopathology-Biotherapy Department (DHU i2B), F-75005, Paris, France;

^bINSERM, UMR_S 959, F-75013, Paris, France; CNRS, FRE3632, F-75005, Paris, France;

^cAP-HP, Groupe Hospitalier Pitié-Salpêtrière, Department of Internal Medicine and Clinical Immunology, F-75013, Paris, France;

^dCentre de Référence des Maladies Auto-Immunes et Systémiques Rares, Centre de Référence des Maladies Auto-Inflammatoires et de l'Amylose, France

^eDepartment of Immunology, UF d'Immunochimie et d'autoimmunité, APHP, Groupe Hospitalier Pitié-Salpêtrière, Paris, France ;

^fDepartment of Biostatistics and Medical Information, INSERM UMR1153 ECSTRRA Team, Hôpital Saint Louis, AP-HP, Paris, France.

Key words: cryoglobulinemia, vasculitis, epidemiology, hepatitis C virus

This paper includes: abstract 228 words, 2272 words, 32 references, 2 tables, 2 figures, 1 supplementary figure

Correspondence should be addressed to: Prof. David Saadoun, AP-HP, Hôpital Pitié-Salpêtrière, Department of Internal Medicine and Clinical Immunology, 83 boulevard de l'hôpital. F-75013, Paris, France

Phone: + (33)(1) 42 17 80 42. Fax: + (33)(1) 42 17 80 80.

E Mail: david.saadoun@aphp.fr

*Co senior

Declaration of interests

David Saadoun has received consulting and lecturing fees from Medimmune, Abbvie, Bristol Meyer Squibb, Celgene, Sanofi, Roche, Servier, Gilead, AstraZeneca and Glaxo Smith Kline. Patrice Cacoub has received consulting and lecturing fees from Abbvie, Astra Zeneca, Bristol-Myers Squibb, Gilead, Glaxo Smith Kline, Janssen, Merck Sharp Dohme, Roche, Servier and Vifor.

Abstract

Objectives: Historically chronic hepatitis C virus (HCV) infection accounted for the majority of mixed cryoglobulinemia (MC). The advent of direct-acting antivirals (DAA) against HCV has dramatically changed the management and the prevalence of chronic HCV infection. We aimed to describe the spectrum of MC in the era of DAA agents.

Methods: We performed a longitudinal cohort study between 2011 to 2018 from a single-centre French university hospital's database of 15 970 patients screened for MC. Epidemiological, clinical and immunological data of MC were recorded. We evaluated the incidence and evolution of MC before and after the era of DAA agents and compared HCV and non-HCV related MC.

Results: Among 742 patients who tested positive for cryoglobulin, 679 [mean age 55.5 years, 54.5% female and 381 (56.1%) with chronic HCV infection] patients with persistent MC were included in the study. 373 (54.9%) had type II and 306 (45.1%) type III cryoglobulin, and 139 (21.5%) had cryoglobulinemia vasculitis (CryoVas). The incidence of MC decreased steadily with 395 and 284 incident cases during 2011-2014 and 2015-2018, respectively. In 2011, the leading cause was chronic HCV infection (62.5% of all MC). Currently, autoimmune diseases [systemic lupus erythematosus (28.9%) and Sjögren's syndrome (10.7%)] are the main cause of MC. The incidence of CryoVas was similar between HCV-and non HCV-related MC.

Conclusion: Direct-acting antivirals have dramatically changed the landscape and the incidence of MC.

Introduction

Cryoglobulins are an abnormal immunoglobulin (Ig) which reversibly precipitates at low temperatures below 37°C. This definition distinguishes cryoglobulins from other cryoproteins and cold agglutinins. Cryoglobulinemia is defined as the persistent presence of cryoglobulins in serum. According to Brouet's classification [1] three subtypes of cryoglobulinemia can be defined: type I made of isolated monoclonal components, type II made of mixed cryoglobulins with a monoclonal component and type III made of mixed polyclonal cryoglobulins. The latter (type II and III) are also known as mixed cryoglobulins (MC). Cryoglobulins can cause systemic vasculitis which is more common in the mixed forms and can be a life-threatening condition [2,3]. Before the era of direct acting antivirals (DAA), chronic hepatitis C virus (HCV) infection accounted for 80-90% of cases of MC [4]. At that time, among the cases of non-related-HCV cryoglobulinemia, B-cell malignancies and connective tissue disease disorders appeared as the leading causes. The remaining non HCV-related cases are classified as having essential cryoglobulinemia [5].

The recent advent of DAA which have the potential to produce sustained virological response rates greater than 95% [6] has dramatically changed the management of chronic HCV infection and HCV-related cryoglobulinemia vasculitis (CryoVas) [7]. In France, DAA are available since 2014 for the treatment of chronic HCV infection. Since the introduction of DAA, data are very scarce on the incidence, etiologies and main characteristics of MC.

Based on a large cohort of patients followed from 2011 to 2018 in a single tertiary university hospital, we aimed to assess the incidence, main etiologies, clinical manifestations and immunological parameters of patients with MC. We also compared HCV-related MC and non HCV-related MC.

1. Material and methods

1.1. Study population and procedures

For this observational longitudinal cohort study, 15 970 patients were screened for cryoglobulinemia at the Pitié-Salpêtrière University Hospital in Paris, France, between 2011 and 2018. Out of this cohort 742 (4.6%) patients were tested positive for cryoglobulin detection and were retrospectively studied (**Figure 1**).

Inclusion criteria for the study was the presence of MC level in serum $>0.05\text{g/L}$ on at least 2 determinations. Cryoglobulins were measured as previously described [8] and classified according to the method described by Brouet et al [1]. All patients with type I cryoglobulinemia or for whom there was no sufficient information available on the etiology of MC were excluded (**Figure 1**). Chart of patients with CryoVas seen between 2011 and 2018 at the Pitié-Salpêtrière University Hospital, Paris, France, were reviewed and demographic information (age at diagnosis, gender, year of diagnosis) was collected. CryoVas cases were defined by clinically active vasculitis with skin, joint, renal, peripheral nerve, central neurologic, digestive, pulmonary and/or cardiac involvement (no histologic evidence needed if the patient had purpura) [9].

1.2. Outcomes

We collected the etiology of MC and clinical manifestations of CryoVas. MC patients were divided into two groups, i.e. HCV-related and non HCV-related MC. The laboratory data collected at the time of initial diagnosis of MC, included cryoglobulin type and serum level (g/L), serum levels of complement C4 fraction (g/L), rheumatoid factor (RF) activity (UI/mL), gamma globulin levels (g/L) as measured by serum protein electrophoresis and monoclonal immunoglobulin levels (g/L) as evaluated by peak integration.

1.3. Statistical analysis

Categorical variables were described as counts (%) and were compared using a χ^2 -test or a Fisher's exact test when appropriate. Continuous variables were described as median and interquartile range and were compared using the Z-score or a Wilcoxon's test when appropriate. All tests were two-sided; p-values lower than 0.05 were considered as indicating a significant association. Analyses were performed using R statistical software, version 3.6.1.

2. Results

2.1. Patient characteristics

Demographic, clinical and immunological features are summarized in **Table 1**. Between 2011 and 2018, a total of 679 consecutive patients met inclusion criteria and were

included of whom 381 (56.1%) had HCV-related MC and 298 (43.9%) had non-HCV-related MC (**Table 1**). Sixty-three patients were excluded because of type I cryoglobulin (n=42) or lack of data (n=21) (**Figure 1**). In the overall cohort, median age at diagnosis was 55 years, the sex ratio (F:M) was 1.2. In the overall cohort, 139 of 679 (21.5%) patients had vasculitis manifestations, and 54.9% and 45.1% had type II and type III mixed cryoglobulin, respectively. 69.9% of patients had elevated rheumatoid factor titers >5UI/mL (25.1 (13.0;76.5) UI/mL) and 52% had low C4 levels <0.16g/L (0.08 (0.04;0.12) g/L). Among type II MC, the monoclonal component was IgM kappa (n=172, 46.2%) IgG kappa (n=50, 13.4%), IgM lambda (n=42, 11.3%) and IgG lambda (n=39, 10.5%). Seventy-three patients (19.6%) had oligoclonal bands.

2.2. Etiologies of mixed cryoglobulinemia and cryoglobulinemia vasculitis

Over the period of 2011 and 2018 chronic HCV infection still appeared as the most frequent etiology accounting for 56% of MC cases. In the remaining 44% of MC the main causes were autoimmune diseases (51.3%), hematological and neoplastic conditions (14.1%), infectious diseases other than chronic HCV infection (10.7%), and essential (23.8%) (**Figure 2**). Among the non HCV-related MC cases, systemic lupus erythematosus (SLE) and Sjögren's syndrome were the most frequent autoimmune conditions (28.9% and 10.7% respectively) and non-Hodgkin's B-cell lymphoma (5.4%) and Waldenström macroglobulinemia (4%) represented the main hematological conditions. HIV infection was the most frequent cause of non HCV infection accounting for 4.4% of cases. Supplementary Figure 1 shows the frequency of the non-HCV etiologies listed by year of diagnosis. We observed the same trend as for MC for the etiologies of CryoVas. Chronic HCV infection appeared as the most frequent etiology accounting for 51.8% of CryoVas. HCV represented 73.9% and 36.4% of CryoVas in 2011 and 2018, respectively. Autoimmune diseases (18.7% of all cases) appeared as the most common cause of non-HCV related CryoVas. Sjögren's syndrome (17.9%) and SLE (11.9%) were the main autoimmune diseases associated with CryoVas. Patients with CryoVas had mainly type II MC (79.1%), and main clinical manifestations included purpura (54.7%), peripheral neuropathy (49.6%), membranoproliferative glomerulonephritis (21.6%) and arthritis (10.1%) (data not shown).

2.3. Incidence and evolution of MC before and after the era of DAA agents

The incidence of MC decreased steadily between 2011 and 2018 with 395 and 284 incident cases during the 2011-2014 and 2015-2018 periods, respectively (**Figure 2**). The evolution of the number of incident new MC cases over the entire period showed 96 cases in 2011, 118 in 2012, 114 in 2013, 67 in 2014, 87 in 2015, 74 in 2016, 72 in 2017 and 51 in 2018. **Figure 2** illustrates the evolution of the incidence and the distribution of MC from 2011 to 2018. In 2011, the leading cause was chronic HCV infection (62.5% of MC). Between 2016 and 2017, we observed a clear decrease in the incidence of HCV-related MC cases (**Figure 2**). In 2018, non-HCV-related MC accounted for 66.7% of all MC cases and autoimmune diseases were the leading cause (35.5% of cases in 2018 vs 17.3% in 2011). Among non-HCV-related MC, SLE and Sjögren's syndrome were the most frequent conditions accounting for 28.9% and 10.7% of cases, respectively. Among autoimmune diseases, SLE and Sjögren's syndrome accounted for 57.9% and 21.1% of cases, respectively. The proportion of HCV-related MC cases over the study period represented 62.5% in 2011, 61.0 % in 2012, 55.3% in 2013, 73.1% in 2014, 59.8% in 2015, 51.4% in 2016, 41.7% in 2017 and 33.3% in 2018. Focusing on CryoVas our results showed that its incidence also decreased between 2011 and 2018 with 93 and 46 new incident cases during the 2011-2014 and 2015-2018 periods, respectively.

2.4. Comparison between HCV and non-HCV related MC

Among all MC positive patients, HCV-related compared to non HCV-related cases were older (55 (38;68) vs 55 (49;65) years, $p < 0.016$) and more frequently male (51.7% vs 37.2%, $p < 0.001$) (**Table 1**). Presence of vasculitis was not statistically different between HCV and non-HCV-related MC. In the overall population, median serum cryoglobulin level was 148 (82;296) mg/l. There was no statistically significant difference in cryoglobulin levels, type of cryoglobulin, monoclonal immunoglobulin levels, RF activity or gamma globulin levels. There was a trend towards lower C4 component levels in patients with HCV-related MC (0.15 (0.08;0.23) vs 0.16 (0.06;0.27) g/L, $p = 0.06$).

2.5. Comparison between type II and type III MC

Data regarding main features of type II and type III MC are summarised in **Table 2**. Compared to type III MC, those with type II MC were significantly older (57 (49;67) vs

51 (39;65) years, $p < 0.001$) and had more frequently CryoVas (30.6 vs 10.1%, $p < 0.001$). SLE was more frequent in type III MC (19.3 vs 7.2%, $p < 0.001$) whereas non-Hodgkin lymphoma and Waldenström disease were more frequent in type II MC (3.5 vs 1.0%, $p = 0.03$ and 3.2 vs 0.0%, $p = 0.002$, respectively). Regarding biological features, type II MC cases had higher cryoglobulin levels (190 (91;424) vs 119 (75;214) mg/l, $p < 0.001$) and lower C4 complement levels (0.15 (0.06;0.24) vs 0.16 (0.10;0.25) g/L, $p = 0.014$). Monoclonal immunoglobulin levels, RF activity and gamma globulin levels were not statistically different between type II and type III MC cases.

3. Discussion

Our study covers the complete spectrum of MC, as the data represent different medical specialties at a university hospital. Between 2011 and 2018, 15 970 patients were screened for the presence of a cryoglobulin. Of these, 679 patients (4.3%) tested positive for MC and were included in this study.

The most striking conclusions drawn by this study are 1) Between 2011 and 2014 HCV-related MC represented the largest cohort. However, from 2016 to 2018, two major epidemiological changes were noted, i.e. non HCV patients represented the largest cohort and the incidence of MC and CryoVas decreased steadily; 2) systemic autoimmune diseases (i.e. SLE and Sjögren syndrome) currently represent the main causes of MC and of CryoVas; and 3) The incidence of CryoVas was similar between HCV and non HCV-related MC.

The development of DAA has dramatically changed the management of chronic HCV infection. Indeed, interferon-free DAA regimens have showed unprecedented high rates of sustained virological response (SVR) of over 90% [10]. With more than 184 million infected patients worldwide and increasing rates of SVR it is expected that by 2030 chronic HCV infection should be confined [11–14]. Up until now, previous studies showed that HCV infection accounted for 80 to 90% of cases of MC [4,15–17]. The prevalence of HCV infection varies depending on the geographical region being highly prevalent (>3.5%) in some parts of Asia, Middle East and Africa and less prevalent (<1%) in several western European countries[18]. Besides liver disease, HCV infection can be involved in many extra-hepatic manifestations mostly driven by chronic immune system stimulation in particular MC. Circulating mixed cryoglobulins are detected in 40 to 60% of chronically infected HCV patients [19,20]. Since it is considered a rare

condition data of prevalence and incidence of MC in the general population are scarce. Our findings suggest that the landscape of MC is changing with HCV-related incident cases dropping continuously since 2011. From 2016 we observed an inversion in the underlying causes of MC and HCV is no longer the leading cause. The prevalence of HCV in our cohort decreased sharply from 62.5% in 2011 to 33.3% in 2018. This is in line with a recent study reporting a prevalence of HCV of 49.5% between 2010 to 2016 in a MC cohort [21]. The same trend was also observed when focusing on patients who were symptomatic (i.e. with CryoVas). As a result, we observed a 1.5 fold decrease in incident MC cases between 2011-2014 and 2015-2018 periods. A previous meta-analysis of extrahepatic manifestations of chronic HCV infection revealed a prevalence of CryoVas of 4.9% [22]. In our cohort, the prevalence of CryoVas was 21.5%. This discrepancy is likely explained by a referral bias in this study conducted in a specialized tertiary medical center.

Previous reports showed a close correlation between virological response and improvement of CryoVas in patients treated with an oral interferon-free regimen[23–27]. Complete disappearance of circulating cryoglobulin was evidenced in up to 50% of cases and DAA therapy reverted autoreactive memory B cell, T follicular helper and Th17 cell expansion and restored regulatory T cell deficiency in HCV-related MC patients [9]. These data provide a mechanistic link between HCV and MC [28].

The present study also provides evidence on the emerging main etiologies of MC. In older reports, 10 to 20% of MC cases unrelated to HCV infection were related to B cell malignancies, non-HCV infectious diseases and autoimmune disorders[29]. Cryoglobulinemia is known to be associated with connective tissue diseases such as SLE and Sjögren's syndrome [30,31] with a prevalence of 10% in SLE and 5 to 20% in Sjögren's syndrome [31,32]. In the present study, autoimmune diseases currently represent the main cause of MC (36.5% of all cases). More specifically, SLE and Sjögren's syndrome are in 2018 the main etiologies of MC and CryoVas, supporting the evolving spectrum of MC with potential important clinical implications.

We identified differences between non-HCV and HCV-related MC patients. Non-HCV related MC patients were younger, more frequently female, and had similar proportion of type II and III MC. They also tended to have higher C4 complement levels compared with those with HCV. However, the prevalence of CryoVas was similar between the two groups.

We acknowledge some limitations in this study. First, this is an observational single-

center tertiary hospital study reviewing only patients that have been tested for cryoglobulin. Secondly, our study does not provide information relative to the institutional or regional incidence of HCV or the frequency of DAAs use. Finally, indications for cryoglobulin testing were not standardized and therefore a selection bias is not excluded.

In conclusion, DAA are changing the landscape of MC with HCV infection no longer being its leading cause. Due to the safety profile and high virological responses rates of DAA regimens in HCV, we speculate that the incidence of HCV-related MC will continue to decline in the upcoming years.

Ethics approval

This retrospective study has been performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. Formal consent was not required.

Writing assistance

None.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Transparency declaration

The manuscript's guarantor (GB) affirms that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted.

Authorship statement

Gonçalo Boleto is the submission's guarantor and takes responsibility for the integrity of the work as a whole, from inception to published article.

Author contributions

All authors were involved in drafting the article or revising it critically for important

intellectual content, and all authors approved the final version to be submitted for publication. Pr. Saadoun had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: David Saadoun, Gonçalo Boleto.

Acquisition of data: Gonçalo Boleto, Pascale Ghillani Dalbin, Lucile Musset.

Analysis and interpretation of data: David Saadoun, Gonçalo Boleto, Patrice Cacoub, Lucie Biard, Guillaume Mulier.

Drafting of the manuscript: David Saadoun, Gonçalo Boleto.

References

- [1] Brouet J-C, Clauvel J-P, Danon F, Klein M, Seligmann M. Biologic and clinical significance of cryoglobulins: A report of 86 cases. *Am J Med* 1974;57:775–88. [https://doi.org/10.1016/0002-9343\(74\)90852-3](https://doi.org/10.1016/0002-9343(74)90852-3).
- [2] Cacoub P, Comarmond C, Domont F, Savey L, Saadoun D. Cryoglobulinemia Vasculitis. *Am J Med* 2015;128:950–5. <https://doi.org/10.1016/j.amjmed.2015.02.017>.
- [3] Ferri C, Sebastiani M, Giuggioli D, Cazzato M, Longombardo G, Antonelli A, et al. Mixed cryoglobulinemia: demographic, clinical, and serologic features and survival in 231 patients. *Semin Arthritis Rheum* 2004;33:355–74.
- [4] Roccatello D, Saadoun D, Ramos-Casals M, Tzioufas AG, Ferverza FC, Cacoub P, et al. Cryoglobulinaemia. *Nat Rev Dis Primer* 2018;4:11. <https://doi.org/10.1038/s41572-018-0009-4>.
- [5] Terrier B, Krastinova E, Marie I, Launay D, Lacraz A, Belenotti P, et al. Management of noninfectious mixed cryoglobulinemia vasculitis: data from 242 cases included in the CryoVas survey. *Blood* 2012;119:5996–6004. <https://doi.org/10.1182/blood-2011-12-396028>.
- [6] Cacoub P, Si Ahmed SN, Ferfar Y, Pol S, Thabut D, Hezode C, et al. Long-term Efficacy of Interferon-Free Antiviral Treatment Regimens in Patients With Hepatitis C Virus-Associated Cryoglobulinemia Vasculitis. *Clin Gastroenterol Hepatol Off Clin Pract J Am Gastroenterol Assoc* 2019;17:518–26. <https://doi.org/10.1016/j.cgh.2018.05.021>.
- [7] Landau D-A, 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. <https://doi.org/10.3899/jrheum.090790>.
- [8] Musset L, Diemert MC, Taibi F, Thi Huong Du L, Cacoub P, Leger JM, et al. Characterization of cryoglobulins by immunoblotting. *Clin Chem* 1992;38:798–802.
- [9] Saadoun D, Pol S, Ferfar Y, Alric L, Hezode C, Si Ahmed SN, et al. Efficacy and Safety of Sofosbuvir Plus Daclatasvir for Treatment of HCV-Associated Cryoglobulinemia Vasculitis. *Gastroenterology* 2017;153:49-52.e5. <https://doi.org/10.1053/j.gastro.2017.03.006>.
- [10] Petta S, Marzioni M, Russo P, Aghemo A, Alberti A, Ascione A, et al. Ombitasvir, paritaprevir, and ritonavir, with or without dasabuvir, plus ribavirin for patients with hepatitis C virus genotype 1 or 4 infection with cirrhosis (ABACUS): a prospective observational study. *Lancet Gastroenterol Hepatol* 2017;2:427–34. [https://doi.org/10.1016/S2468-1253\(17\)30048-1](https://doi.org/10.1016/S2468-1253(17)30048-1).
- [11] Flemming JA, Kim WR, Brosgart CL, Terrault NA. Reduction in liver transplant wait-listing in the era of direct-acting antiviral therapy. *Hepatology* 2015:804–12. [https://doi.org/10.1002/hep.28923@10.1002/\(ISSN\)1527-3350\(CAT\)VirtualIssues\(VI\)HepatologyHonorRoleEditorsChoice](https://doi.org/10.1002/hep.28923@10.1002/(ISSN)1527-3350(CAT)VirtualIssues(VI)HepatologyHonorRoleEditorsChoice).
- [12] Polaris Observatory HCV Collaborators. Global prevalence and genotype distribution of hepatitis C virus infection in 2015: a modelling study. *Lancet Gastroenterol Hepatol* 2017;2:161–76. [https://doi.org/10.1016/S2468-1253\(16\)30181-9](https://doi.org/10.1016/S2468-1253(16)30181-9).
- [13] Belli LS, Berenguer M, Cortesi PA, Strazzabosco M, Rockenschaub S-R, Martini S, et al. Delisting of liver transplant candidates with chronic hepatitis C after viral eradication: A European study. *J Hepatol* 2016;65:524–31.

- <https://doi.org/10.1016/j.jhep.2016.05.010>.
- [14] Razavi H, Robbins S, Zeuzem S, Negro F, Buti M, Duberg A-S, et al. Hepatitis C virus prevalence and level of intervention required to achieve the WHO targets for elimination in the European Union by 2030: a modelling study. *Lancet Gastroenterol Hepatol* 2017;2:325–36. [https://doi.org/10.1016/S2468-1253\(17\)30045-6](https://doi.org/10.1016/S2468-1253(17)30045-6).
- [15] Ferri C, Greco F, Longombardo G, Palla P, Moretti A, Marzo E, et al. Association between hepatitis C virus and mixed cryoglobulinemia [see comment]. *Clin Exp Rheumatol* 1991;9:621–4.
- [16] Ferri C, Greco F, Longombardo G, Palla P, Moretti A, Marzo E, et al. Antibodies to hepatitis C virus in patients with mixed cryoglobulinemia. *Arthritis Rheum* 1991;34:1606–10.
- [17] Cacoub P, Poynard T, Ghillani P, Charlotte F, Olivi M, Piette JC, et al. Extrahepatic manifestations of chronic hepatitis C. MULTIVIRC Group. Multidepartment Virus C. *Arthritis Rheum* 1999;42:2204–12. [https://doi.org/10.1002/1529-0131\(199910\)42:10<2204::AID-ANR24>3.0.CO;2-D](https://doi.org/10.1002/1529-0131(199910)42:10<2204::AID-ANR24>3.0.CO;2-D).
- [18] 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. *Hepatol Baltim Md* 2013;57:1333–42. <https://doi.org/10.1002/hep.26141>.
- [19] Ramos-Casals M, Stone JH, Cid MC, Bosch X. The cryoglobulinaemias. *Lancet Lond Engl* 2012;379:348–60. [https://doi.org/10.1016/S0140-6736\(11\)60242-0](https://doi.org/10.1016/S0140-6736(11)60242-0).
- [20] Desbois AC, Cacoub P, Saadoun D. Cryoglobulinemia: An update in 2019. *Jt Bone Spine Rev Rhum* 2019. <https://doi.org/10.1016/j.jbspin.2019.01.016>.
- [21] Kolopp-Sarda MN, Nombel A, Miossec P. Cryoglobulins today: detection and immunological characteristics of 1600 positive samples out of 13000 patients over 6 years. *Arthritis Rheumatol n.d.;0*. <https://doi.org/10.1002/art.41003>.
- [22] Younossi Z, Park H, Henry L, Adeyemi A, Stepanova M. Extrahepatic Manifestations of Hepatitis C: A Meta-analysis of Prevalence, Quality of Life, and Economic Burden. *Gastroenterology* 2016;150:1599–608. <https://doi.org/10.1053/j.gastro.2016.02.039>.
- [23] Gragnani L, Fabbrizzi A, Triboli E, Urraro T, Boldrini B, Fognani E, et al. Triple antiviral therapy in hepatitis C virus infection with or without mixed cryoglobulinaemia: a prospective, controlled pilot study. *Dig Liver Dis Off J Ital Soc Gastroenterol Ital Assoc Study Liver* 2014;46:833–7. <https://doi.org/10.1016/j.dld.2014.05.017>.
- [24] Saadoun D, Thibault V, Si Ahmed SN, Alric L, Mallet M, Guillaud C, et al. Sofosbuvir plus ribavirin for hepatitis C virus-associated cryoglobulinaemia vasculitis: VASCUVALDIC study. *Ann Rheum Dis* 2016;75:1777–82. <https://doi.org/10.1136/annrheumdis-2015-208339>.
- [25] Sise ME, Bloom AK, Wisocky J, Lin MV, Gustafson JL, Lundquist AL, et al. Treatment of hepatitis C virus-associated mixed cryoglobulinemia with direct-acting antiviral agents. *Hepatol Baltim Md* 2016;63:408–17. <https://doi.org/10.1002/hep.28297>.
- [26] Gragnani L, Visentini M, Fognani E, Urraro T, De Santis A, Petracchia L, et al. Prospective study of guideline-tailored therapy with direct-acting antivirals for hepatitis C virus-associated mixed cryoglobulinemia. *Hepatol Baltim Md* 2016;64:1473–82. <https://doi.org/10.1002/hep.28753>.
- [27] Saadoun D, Pol S, Ferfar Y, Hezode C, Ahmed SNS, Alric L, et al. Sofosbuvir

- plus daclatasvir for hepatitis C virus-cryoglobulinemia vasculitis (HCV-CryoVas): VASCUVALDIC 2 Study. *J Hepatol* 2017;66:S56–7. [https://doi.org/10.1016/S0168-8278\(17\)30375-6](https://doi.org/10.1016/S0168-8278(17)30375-6).
- [28] Bonacci M, Lens S, Mariño Z, Londoño M-C, Rodríguez-Tajes S, Sánchez-Tapias JM, et al. Long-Term Outcomes of Patients With HCV-Associated Cryoglobulinemic Vasculitis After Virologic Cure. *Gastroenterology* 2018;155:311-315.e6. <https://doi.org/10.1053/j.gastro.2018.04.024>.
- [29] Terrier B, Marie I, Lacraz A, Belenotti P, Bonnet F, Chiche L, et al. Non HCV-related infectious cryoglobulinemia vasculitis: Results from the French nationwide CryoVas survey and systematic review of the literature. *J Autoimmun* 2015;65:74–81. <https://doi.org/10.1016/j.jaut.2015.08.008>.
- [30] Ramos-Casals M, Cervera R, Yagüe J, García-Carrasco M, Trejo O, Jiménez S, et al. Cryoglobulinemia in primary Sjögren’s syndrome: prevalence and clinical characteristics in a series of 115 patients. *Semin Arthritis Rheum* 1998;28:200–5.
- [31] García-Carrasco M, Ramos-Casals M, Cervera R, Trejo O, Yagüe J, Sisó A, et al. Cryoglobulinemia in systemic lupus erythematosus: prevalence and clinical characteristics in a series of 122 patients. *Semin Arthritis Rheum* 2001;30:366–73.
- [32] Tzioufas AG, Manoussakis MN, Costello R, Silis M, Papadopoulos NM, Moutsopoulos HM. Cryoglobulinemia in autoimmune rheumatic diseases. Evidence of circulating monoclonal cryoglobulins in patients with primary Sjögren’s syndrome. *Arthritis Rheum* 1986;29:1098–104.

Table 1

	Population (n=679)	Non-HCV (n=298)	HCV (n=381)	P-value
Age, median (IQR)	55(46;66)	55(38;68)	55(49;65)	0.016
Male gender	308 (45.4)	111 (37.2)	197 (51.7)	<0.001
Etiologies				
HCV	381 (56.1)		381 (100.0)	
AUTOIMMUNE	153 (22.5)	153 (51.3)		
SLE	86 (12.7)	86 (28.9)		
Sjögren's syndrome	32 (4.7)	32 (10.7)		
Mixed connective tissue disease	6 (0.9)	6 (2.0)		
Antiphospholipid syndrome	6 (0.9)	6 (2.0)		
ANCA vasculitides	6 (0.9)	6 (2.0)		
Myositis	4 (0.6)	4 (1.3)		
HEMATOLOGICAL/ NEOPLASTIC	42 (6.2)	42 (14.1)		
Non-Hodgkin lymphoma	16 (2.4)	16 (5.4)		
Waldenström macroglobulinemia	12 (1.8)	12 (4.0)		
Chronic lymphocytic leukemia	4 (0.6)	4 (1.3)		
MGUS	3 (0.4)	3 (1.0)		
Solid neoplasm	3 (0.4)	3 (1.0)		
INFECTIOUS	32 (4.7)	32 (10.7)		
HIV	13 (1.9)	13 (4.4)		
Malaria	3 (0.4)	3 (1.0)		
ESSENTIAL	71 (10.5)	71 (23.8)		
Cryoglobulin levels (mg/L), median (IQR)	148 (82;296)	97 (67;168)	214 (113;376)	0.92
Type of cryoglobulin				0.18
II	373 (54.9)	155 (52.0)	218 (57.2)	
III	306 (45.1)	143 (48.0)	163 (42.8)	
Monoclonal Ig (g/L), median (IQR)	4.7 (2.8;8.8)	5.3 (3.0;11.4)	4.4 (2.8;6.9)	0.68*
C4 level (g/L), median (IQR)	0.15 (0.07;0.25)	0.16 (0.06;0.27)	0.15 (0.08;0.23)	0.06
Rheumatoid factor (UI/mL), median (IQR)	30.0 (6.7;109.1)	20.8 (5.0;97.9)	42.9 (11.4;112.1)	0.9
Gammaglobulin levels (g/L), median (IQR)	13.9 (10.5;18.8)	13.9 (10.3;18.8)	13.9 (11.0;18.4)	0.48
Cryoglobulinemia vasculitis	139 (21.5)	67 (22.8)	72 (20.4)	0.46
* : Wilcoxon's rank sum test				

Data are n (%) unless otherwise stated.

Abbreviations: IQR, interquartile range; HCV, hepatitis C virus; MC, mixed cryoglobulinemia; SLE, systemic lupus erythematosus; ANCA, antineutrophil cytoplasmic antibody; MGUS, monoclonal gammopathy of undetermined significance; HIV, human immunodeficiency virus; Ig, immunoglobulin.

Table 2

	Type 2 (n=373)	Type 3 (n=306)	P-value
Age, median (IQR)	57(49;67)	51(39;65)	<0.001
Male gender	180 (48.3)	128 (41.8)	0.09
Etiologies			
HCV	218 (58.4)	163 (53.3)	0.18
AUTOIMMUNE			
SLE	27 (7.2)	59 (19.3)	<0.001
Sjögren's syndrome	21 (5.6)	11 (3.6)	0.21
Mixed connective tissue disease	3 (0.8)	3 (1.0)	1**
Antiphospholipid syndrome	3 (0.8)	3 (1.0)	1**
ANCA vasculitides	2 (0.5)	4 (1.3)	0.42**
Myositis	1 (0.3)	3 (1.0)	0.33**
HEMATOLOGICAL/NEOPLASTIC			
Non-Hodgkin lymphoma	13 (3.5)	3 (1.0)	0.03
Waldenström macroglobulinemia	12 (3.2)	0 (0.0)	0.002
Chronic lymphocytic leukemia	3 (0.8)	1 (0.3)	0.63**
MGUS	3 (0.8)	0 (0.0)	0.26**
Solid neoplasm	2 (0.5)	1 (0.3)	1**
INFECTIOUS			
HIV	9 (2.4)	4 (1.3)	0.3
Malaria	1 (0.3)	2 (0.7)	0.59**
ESSENTIAL			
Cryoglobulin levels (mg/L), median (IQR)	190 (91;424)	119 (75;214)	<0.001
Monoclonal Ig (g/L), median (IQR)	43 5.4(2.5;9.7)	3 4.1(3.7;4.2)	0.49*
C4 level (g/L), median (IQR)	0.15 (0.06;0.24)	0.16 (0.10;0.25)	0.014
Rheumatoid factor (UI/mL), median (IQR)	31.0 (5.8;138.0)	29.2 (7.3;71.2)	0.38
Gammaglobulin levels (g/L), median (IQR)	13.6 (10.2;20.0)	14.6 (11.2;17.7)	0.95
Cryoglobulinemia vasculitis	110 (30.6)	29 (10.1)	<0.001
* : Wilcoxon's rank sum test			
** : Fisher's exact test			

Data are n (%) unless otherwise stated.

Abbreviations: IQR, interquartile range; MC, mixed cryoglobulinemia; HCV, hepatitis C virus; SLE, systemic lupus erythematosus; ANCA, antineutrophil cytoplasmic antibody; MGUS, monoclonal gammopathy of undetermined significance; HIV, human immunodeficiency virus; Ig, immunoglobulin.

Legends

Table 1

Main characteristics of patients with mixed cryoglobulinemia.

Table 2

Comparison between patients with type II and type III mixed cryoglobulinemia.

Figure 1

Flow-chart of patients included in the study.

Figure 2

Evolution of the etiologies of mixed cryoglobulin (MC) over the period 2011-2018.

(A) Decrease in the proportion of patients with HCV-related MC from 2014 with non-HCV-related MC representing the majority of cases from 2016;

(B) Decrease in the incidence of overall MC cases. From 2016, non-HCV patients represented the largest population of MC.

HCV, hepatitis C virus.

Supplementary Figure 1

Frequency of the non-HCV-related MC etiologies by year over the period 2011-2018.

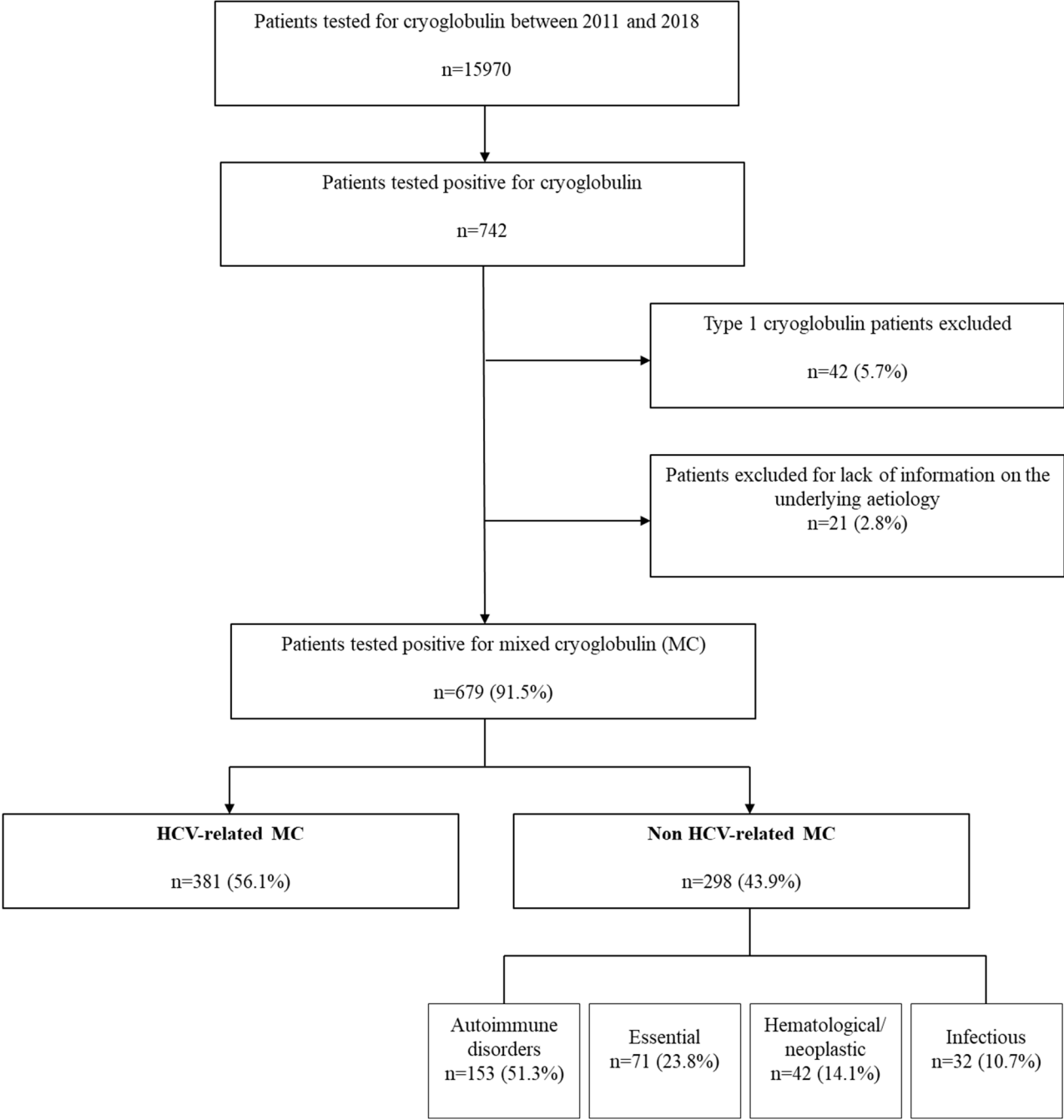


Figure 2

