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A prospective study of DAA Effectiveness and Relapse Risk in HCV Cryoglobulinemic Vasculitis by the Italian PITER Cohort

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List of Abbreviations

MC, Mixed cryoglobulinemia; HCV, Hepatitis C Virus; CV, Cryoglobulinemic Vasculitis; SVR, sustained virologic response; DAA, Direct Acting Antiviral; CGs, cryoglobulins; Ig, Immunoglobulin; RF, rheumatoid factor; IFN, Interferon; PITER, Italian Platform for the Study of Viral Hepatitis Therapy; eCRF, electronic Case Report Form; EOT, end of treatment; FU, follow-up; CR, Complete Clinical Response; FCR, Full Complete Clinical Response; PR, Partial Clinical Response; NR, No Clinical Response; VAS, Visual Analog Scale; SD, standard deviation; CI, Confidence Interval; HR, Hazard ratio, OR, Odd ratio; GFR, glomerular filtration rate.

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Abstract

Background & Aims: Mixed cryoglobulinemia (MC) is the most common Hepatitis C Virus (HCV) extrahepatic manifestation. We aimed to prospectively evaluate the Cryoglobulinemic Vasculitis (CV) clinical profile after a sustained virologic response (SVR) over a medium-long term period.

Approach: Direct Acting Antiviral (DAA) treated cryoglobulinemic patients, consecutively enrolled in the multicentric PITER cohort, were prospectively evaluated. Cumulative incidence, Kaplan Meier curves were reported for response, clinical deterioration or relapse and survival free rates. Cox regression analysis evaluated factors associated with different outcomes.

Results: A clinical response was reported in at least one follow-up point for 373 of 423 (88%) CV patients who achieved a SVR. Clinical response increased over time with a 76% improvement rate at month 12 after the end of treatment. A Full Complete Response (FCR) was reached by 164 (38.8%) patients in at least one follow-up point. CV clinical response fluctuated, with some deterioration of the initial response in 49.6% of patients (median time of deterioration: 19 months). In patients who achieved a FCR and had an available follow-up (137 patients) a relapse was observed in 13%; it was transient in 66.7% of patients. The rate of patients without any deterioration was 58% and 41% at 12 and 24 months, respectively. After achieving a SVR, a clinical non-response was associated with age and renal involvement; a clinical deterioration/relapse was associated with high pre-treatment rheumatoid factor values and a FCR was inversely associated with age, neuropathy, and high cryocrit levels.

Conclusions: In CV patients, HCV eradication may not correspond to a persistent clinical improvement and clinical response may fluctuate. This implies an attentive approach to post-SVR evaluation through prognostic factors and tailored treatment.

Introduction

Hepatitis C virus (HCV) infection is the major cause of liver-related morbidity and is increasingly recognized as a trigger of B cell lymphoproliferative disorders such as Mixed Cryoglobulinemia (MC) and non-Hodgkin's lymphoma (1-5).

MC is characterized by intravascular immune complexes named cryoglobulins (CGs) (6). Mixed CGs are immune complexes that reversibly precipitate when the temperature is below 37°C and consist of polyclonal immunoglobulins (Ig)Gs and IgMs with rheumatoid factor (RF) activity (7). IgMs are mono- or oligo-clonal in type II MC or polyclonal in type III MC (7-9).

MC may not have any clinical symptoms; however, it can cause a wide spectrum of clinical presentations including skin lesions, arthralgia, peripheral neuropathy and single or multiple organ damage, which are the clinical manifestations of systemic vasculitis or the so called Cryoglobulinemic Vasculitis (CV), involving small/medium-vessels, (1, 3, 10-12). CV is both an autoimmune and a lymphoproliferative disease. Even though it is clinically benign, it could evolve into lymphoma in a severe presentation (4, 6, 13).

It has been recently reported that HCV is no longer the main cause of CV in France (14), whereas HCV has still been reported the aetiologic agent in most patient with MC in different clinical centers in Italy. Most cryoglobulinemic patients are HCV positive (70-90%) and conversely 40-60% of HCV infected patients produce CGs of whom 5-30% have CV (3). Due to these anomalies in the clinical manifestations and in the diagnostic criteria, MC and CV remain elusive conditions.

Although the therapeutic approach to CV patients includes several options, such as steroids, plasmapheresis, and rituximab, etiological therapy is the first-line choice in HCV-CV patients. In the past, this therapy was based on the use of Interferon (IFN), despite consistent side effects and intolerances (15, 16). The treatment of HCV infection has undergone a substantial advancement with the introduction of new direct-acting antivirals (DAAs).

Patients with HCV-CV have high rates of clinical remission after treatment with DAAs (17-20), although, in some patients, symptoms may persist or reappear after a transient clinical response (21-27).

The aim of this study was to evaluate the clinical presentation of patients with MC in Hepatology centers in Italy and prospectively evaluate medium-long term clinical profile in patients with CV, in whom HCV was successfully eradicated. The final goal was to enhance our knowledge pertaining to the natural history of cryoglobulinemia after a sustained virological response (SVR) in order to better define patients in whom a continuous monitoring and treatment work up is required.

Methods

Patients

The study population consisted of patients with chronic HCV infection consecutively enrolled in *Piattaforma Italiana per lo studio della Terapia delle epatiti ViRali* (PITER) from about 60 hepatology centers distributed throughout the entire Italian territory (28) and could be considered a representative sample of patients with chronic HCV infection in care in Italy. In this prospective analysis, all patients with cryoglobulinemia were consecutively enrolled, either with CV according to standard criteria (2, 29) or without (only with laboratory findings: MC). We subsequently evaluated patients with CV following the DAA IFN-free regimen during the period from 2015 to 2019.

For each patient, the main data regarding MC/CV were prospectively collected as reported by the prescribing clinicians following the different steps of DAA treatment in a dedicated section of the electronic Case Report Form (eCRF). Specifically, dedicated information regarding the main symptoms (i.e., purpura, asthenia, arthralgia, neuropathy, renal involvement, xerostomia/xerophthalmia, Raynaud phenomenon and ulcers) was recorded. Laboratory findings i.e., cryocrit levels, RF, and C4 complement values were collected, when available, following DAA treatment [pretreatment, end of treatment (EOT) and at different points of the follow-up (FU), depending on the time of the real practice scheduled check-ups at each clinical center]. MC type (II or III) was also recorded, when available.

Liver damage was assessed by fibroscan (transient elastography) (30). The severity of liver disease was classified as "F0-F3" if the stiffness score was equal to or lower than 12.5 kPa and as "F4 Cirrhosis" if it was higher than 12.5 kPa or if there were signs of liver cirrhosis (signs of portal hypertension) (31). HIV or HBV coinfected patients were excluded.

Outcomes

Following HCV eradication, an evaluation of clinical outcomes related to CV was prospectively conducted in patients with at least a 12-month FU after a SVR.

The clinical outcome was defined as previously described (17, 18). Complete Clinical Response (CR) was assessed when all baseline clinical manifestations had improved, with the distinction of a Full Complete Clinical Response (FCR) when all the symptoms disappeared ("*restitution ad integrum*"), a Partial Clinical Response (PR) with an improvement in at least half of the baseline symptoms and No Clinical Response (NR) in the remaining cases. Therefore, the symptoms were

recorded considering the modifications (improvement or worsening) compared to the previous check-up at every FU point. Starting from the end of treatment, in patients who achieved a SVR, at least one follow-up was available up to the 12-month and then in a different subgroup analysis, a follow-up time after the first observation of a clinical response allowed for the evaluation of the outcome behavior over time.

CV symptoms were assessed as has been reported in previous studies (17, 18). Briefly, purpura was classified in 4 semi-quantitative grades: 0 (absence), + (limited and fluctuating on the lower limbs), ++ (diffuse and persistent on the lower limbs), +++ (involvement of the trunk and the lower limbs); leg ulcer response was complete with complete healing, a reduction of at least 25% in diameter was considered an improvement and a lower reduction or a worsening corresponded to a non-response. Arthralgia, weakness and sicca syndrome were measured through a patient-scored Visual Analog Scale (VAS) (range, 0-100). Neuropathic symptoms, including both paraesthesias/pain and motor deficit, were also assessed by VAS and, when possible, by electromyography. Kidney function was evaluated according to serum creatinine and proteinuria. The questionnaire was completed by 87% of patients with CV; in the remaining (13% of patients) the only qualitative evaluation (improvement or deterioration or no changes) was reported for each symptom at baseline and at each point of follow-up. The same specialist from each clinical center evaluated each patient during different follow-up appointments. The evaluation was conducted having the reference as a baseline, prior to therapy evaluation for the first appointment after the end of treatment. Subsequently, the data reported were compared with the previous clinical data. Differences in the scores were evaluated for each patient and were reported as improved when an increasing positive value was detected and as deteriorated when a negative value was detected.
The term “clinical relapse” refers to the reappearance of the syndrome in patients who had previously reached a FCR, whereas the terms “clinical deterioration” and “clinical improvement” denote a worsening or improvement in one or more symptoms respectively.

Statistical Analysis

Quantitative variables were reported as mean ± standard deviation (SD) or median and Interquartile range (Q1-Q3), while categorical variables were summarized by number and percentage. Chi square test or Fisher exact test and t- Test or Mann Whitney test were used to

compare, respectively, categorical and quantitative variables between symptomatic and non-symptomatic patients.

Survival analysis was used to examine clinical response and clinical relapse after the first clinical response, during the FU. Patients who did not experience an event at the end of the period of observation were considered censored. The cumulative incidence curve was represented for clinical response and cumulative incidence and relative confidence interval at 95% (CI95%) were reported at different time points. The Kaplan Meier curve was reported for clinical relapse and survival free rates and relative CI95% were described at different time points.

Univariate Cox regression was used to evaluate factors associated with FCR without deterioration or relapse after the first clinical response. Only variables with a p value <0.15 in univariate models were considered in the multivariable analysis. Stepwise Cox regression (p entry and exit=0.15) was implemented in the analysis of FCR without deterioration/relapse, considering the reduction of sample size and the number of events caused by missing values. Hazard ratio (HR) and relative CI95% were reported.

Mixed models were implemented to evaluate laboratory data over time considering repeated measures for the same patient. Estimated mean and relative CI95% were reported. The p value in comparison with the baseline value was adjusted by Dunnet correction.

Logistic regression was implemented to evaluate the risk factor associated to NR at the EOT. Only variables with a p value <0.15 in univariable models were considered in the multivariable analysis. Odd ratio (OR) and relative CI95% were reported.

SAS 9.4 was used for all analyses and a p value <0.05 was considered statistically significant.

Ethics

The study was conducted in accordance with the guidelines of the Declaration of Helsinki and the principles of Good Clinical Practice. The study protocol was approved by the Ethics Committee of the Istituto Superiore di Sanità (Prot. CE/13/389 - 23/07/2013) and by the local Ethics committees from each clinical center. Patients' data were evaluated via an anonymous analysis, adopting codes generated from the electronic case report forms. All patients provided written informed consent to participate in the study.

Results

CV characteristics at enrollment

Out of 11,871 consecutively enrolled patients, the cryoglobulinemic status was only available in 28.5% of patients (n=3,390). Among these, 1,255 (37%) patients had circulating CGs at enrollment: these included 523 (41.7 %) patients with symptoms i.e., patients with CV (as reported below) and the remaining 732 (58.3%) were symptomless (MC). CGs type was available in 937 patients and consisted of type II in 67% and type III in 33%.

The symptoms of CV patients at enrollment (regardless of previous symptomatic status) were mostly represented, as expected, by components of the classical triad, purpura, asthenia and arthralgia, followed by neuropathy and sicca syndrome (xerostomia/xerophthalmia); each symptom was present in a percentage of patients ranging from 69% to 95%. Sixty-four (12.2%) patients had renal involvement (from proteinuria and hematuria at urinalysis to a frank reduction in glomerular filtration rate (GFR).

DAA treatment virological response

Among the 1,255 cryoglobulinemic patients, 1,204 (96%) achieved the SVR to a first-line DAA therapy. Out of the 1,255 evaluated patients, 51 (4%) did not appear to be responders (relapse or breakthrough during the first-line DAA therapy), 19 of whom (1.5 %) underwent a second-line DAA therapy, while the remaining 32 (2.5%) patients were lost upon FU. Patients who failed to eradicate HCV by the first-line DAA therapy, underwent a second-line DAA therapy and all, but 2 out of 19 (10.5%), achieved a SVR. Overall, considering first- and second-line therapy, 1,221 reached a SVR.

There were no differences in the SVR rate achieved from the first-line DAA therapy between CV and MC patients (data not shown).

Demographic and clinical characteristics of patients with MC

Out of 1,221 patients who achieved a SVR (after the first-or second-line DAA treatment,), the main pre-treatment demographic and clinical data of CV and MC patients are shown in Table 1.

No difference in age was observed between the two groups. The female gender was more prevalent among patients with CV. The distribution of fibrosis stage was different between CV and MC patients (p=0.013) and the prevalence of cirrhosis was higher in patients with MC.

CV clinical response

A flow chart of the evaluated patients in each sub analysis has been reported in Figure 1. Data concerning clinical response following a SVR were available for 423 CV patients. The median (Q1-Q3) follow-up time was 15 (13-27) months. At the EOT, among 423 patients who achieved the SVR, 57% reached a clinical response (28% a PR, 17% a CR, and 12% a FCR) and 43% were classified as NR.

It was observed that a CV clinical response (FCR+CR+PR) was reported during at least one point of the FU in 373 out of 423 (88%) CV patients and, in particular, a FCR (complete disappearance of all the manifestations) was reached, during at least one point of the FU, by 164 (38.8%) patients.

Figure 2 (A) shows the curve of the first time in which a clinical response (either CR, FCR or PR) was observed. After the SVR assessment, the clinical response rate at month 12 of the FU was 76% and the median time of the first clinical response was 3 months. Pertaining to the different degrees of clinical response, improvements increased over time (second and third year of the FU).

Considering the 288 SVR patients with data corresponding to more than one FU point after the first observation of a clinical response (and consequently allowing for the evaluation of the outcome behavior over time), a clinical deterioration of the initial response or relapse was recorded in 143 patients (49.6%). In patients with fluctuations in the clinical pattern the median time of deterioration in the clinical status was 19 months. The rate of patients without deterioration was 58% at 12 months and 41% at 24 months [Figure 2 (B)].

Symptoms that persisted more frequently 2 years after viral eradication were arthralgia (45%) fatigue (41%) neuropathy (38%) and sicca syndrome (37%).

Out of 164 patients who achieved a FCR (at one point of FU), 137 had an available FU after the achievement of the FCR. A relapse was observed in 13% of patients (18 patients) at least, at one point of FU after a FCR. Interestingly, 9 out of these 18 patients were further evaluated after the clinical relapse, demonstrating that the latter was transient in most cases (6/9, 66.7%), with only 3 out of 9 (33.3%) patients who maintained the NR degree of clinical response during the whole FU.

CV immunological response

The modifications of the main CV laboratory data before and after antiviral therapy are reported in Figure 3. A significant improvement was observed at 24 weeks after the EOT increasing even further during the long-term FU.

Pre-treatment factors potentially associated with a Clinical Response at the end of treatment

As shown in Table 2, out of 309 patients for whom it was possible to assess clinical response at the end of DAA treatment, factors independently associated with a non-clinical response at the EOT from the multivariate analysis included age (OR 95% CI 1.02: 1-1.04; p=0.039) and renal involvement (OR 95% CI =1.79 0.96-3.36; p=0.05).

Pre-treatment factors potentially associated with a Full Complete Response without clinical deterioration or relapse

To evaluate FCR without relapse, out of the 423 patients, 54 patients reached a FCR, but did not undergo a clinical evaluation after this endpoint. Among the 369 patients evaluated, 62 (16.8%) reached a persistent FCR without clinical deterioration during the FU. As shown in Table 3, female sex, advanced age, purpura at the time of admission to the study (pretreatment), arthralgia, neuropathy and higher cryocrit values lowered the probability of maintaining a FCR without clinical deterioration or relapse. After stepwise regression, age, neuropathy and high cryocrit levels remained in the model as independently inversely associated with the outcome (maintaining the FCR without a clinical deterioration (Table 3).

Pre-treatment factors associated with a clinical deterioration or relapse after clinical response

Out of 288 patients who achieved a clinical response (FCR+CR+PR) and had at least one FU point after the clinical response, 143 (49.6%) showed a successive clinical deterioration or relapse. As shown in Table 4, by univariate Cox regression analysis, a significant association between clinical deterioration or relapse and RF values and the presence of sicca syndrome before treatment was observed. The multivariable model showed that pretreatment high RF values represented an independent prognostic index of clinical deterioration or relapse during the FU. The distribution of

pretreatment RF values in CV patients with or without clinical relapse after DAA-based therapy is reported in Figure 4.

Discussion

This is a nation-wide, multi-centric prospectic study, evaluating HCV cryoglobulinemic patients both before and after DAA-based treatment, distinguishing patients with and without vasculitis. This may be of interest especially to the hepatologists involved in the treatment and monitoring of all cryoglobulinemic HCV patients, complessively ranging from 40 to 60% of HCV-positive patients (3). In fact, a dedicated approach focusing on the diagnosis and impact of MC treatment has been included in PITER, which is a prospective cohort of consecutive patients admitted to the most important Italian Hepatology centers as in/out patients prior to antiviral treatment (27).

The underestimation of MC status confirms previous *ad interim* data, collected during the PITER enrollment phase, showing a high real-life variability in the diagnostic approach to MC (32). Most of the enrolling Hepatology centers used to perform MC tests only when CV was clinically evident. Furthermore, cryo testing, requiring special blood sample management, is frequently inadequate.

Regarding the characteristics of cryoglobulinemic patients, the higher prevalence of the female sex and high median age confirmed previously reported data (10, 29, 33, 34).

Although the association between HCV and cryoglobulinemia is widely recognized, its relationship with liver disease is still unclear and there are some contradictory results between the data reported in this analysis and previously published ones (35). In this study, including both CV and MC patients, advanced fibrosis was associated with MC. Some differences in the clinical characteristics of patients referred to different specialists (e.g., Rheumatologists, Hematologists and Nephrologists), could play a role in determining the differences in liver fibrosis stage distribution.

Pertinent to virological response, the SVR rate observed in patients with MC was similar to the one observed by other authors (18, 21, 36) during the same time-frame in non-cryoglobulinemic HCV patients from the PITER cohort (37).

Concerning the clinical outcome, the overly enthusiastic attitude of the first studies on outcomes following DAA therapy, has been hampered by recent studies with longer post-SVR monitoring revealing that the clinical management of SVR-CV patients is more complex than expected (17-20, 23, 25, 38-40). In fact, the persistence of symptoms and/or a later recurrence after a transient clinical response has recently been described by other authors (19, 21-23, 25, 27, 40).

Therefore, in our study, special attention was paid to the characterization of CV clinical response over time following a SVR, with the distinction of 3 degrees of a clinical response, and the evaluation of the kinetics of clinical improvement/deterioration during different FU points.
A clinical response to some degree (FCR, CR and PR) was observed in 57% of patients at the end of treatment which increased to 88% at one point of the FU, whereas 12%, remained NR as no improvement in at least half of the symptoms was observed at the end of the second–third year of the FU.

The design of the present study was also meant to indicate the time when the first clinical response could be expected. This is clinically relevant since CV patients often did not show a clinical improvement at the end of treatment, but later, with the first amelioration starting about 3 months after the EOT (median time: 9 months). In addition, about 50% of patients experienced a further improvement in the first and second year after viral eradication. However, a deterioration in the initial response was also observed in 49.6% of patients in a median time of around 19 months.

Interestingly, a FCR rate was persistent without deterioration only in 16.8% of patients who achieved the FCR during the FU. This implies that, after viral eradication, the persistence of some or most pretreatment symptoms should be considered as not infrequent.

Apart from modulations of the clinical responses, as worsening or improvement during the FU, we also observed a consistent percentage (about 13%) of clinical relapses in patients who had achieved a FCR. This percentage was not far from previous observations, even if performed in different settings (22, 24, 26, 41). For the first time, this study made it possible to highlight that relapse was transitory in about 70% of cases. This stresses the usefulness of accurate monitoring over time for these patients, possibly with the aid of prognostic predictors in order to avoid inappropriate therapeutic strategies.

Concerning laboratory data, a close association between SVR and cryocrit lowering, RF decreasing as well as an increase in C4 values, was observed after treatment thus confirming previous studies (17, 18, 20, 38).

Regarding potential clinical outcome predictors, at the end of the treatment, the multivariate logistic analysis showed that age and the presence of nephropathy were independent prognostic factors of the non-response. This observation, already suggested by previous studies (18, 21, 25, 39), may be correlated with more advanced vasculitis and with non-reversible organ damage.

Focusing on the factors potentially associated with a FCR after treatment, age and neuropathy emerged as independent negative prognostic factors, while among laboratory data, a higher baseline cryocrit level was an independent predictor of non-response, stressing the need to treat asymptomatic patients before the development of organ damage (e.g., neurological and/or renal).

In relation to the risk of long-term clinical deterioration or relapse in patients who achieved a clinical response, high baseline RF values emerged as an independent prognostic index for clinical relapse during the FU, confirming previous results in less numerous cohorts (27). This finding has a pathophysiological meaning as RF molecules are produced by the same B cells whose clonal expansion is considered the basis of cryoglobulinemia (27, 42). It is also an indirect confirmation of the possible maintenance of the pathogenic clones in CV patients after a SVR (27, 43). Data relating to RF are clinically relevant taking into account that it can easily be assessed through a non-invasive peripheral blood draw, whereas performing a reliable cryocrit is difficult in clinical practice (44).

Apart from the risk of clinical relapse, a long-term FU of CV patients maintaining circulating cryoglobulins is useful when considering the possible evolution into a frank non-Hodgkin lymphoma.

Our study has some limitations. We acknowledge the potential center-related underestimation of MC prevalence among individuals with chronic HCV infection in Italy. In addition, the potential non uniformity in reporting the subjective data from different centers and for all enrolled patients could have impacted the evaluation of clinical response, however the semiquantitative evaluation of the main symptoms could have reduced this bias.

In conclusion, the prospective analysis of DAA-treated cryoglobulinemic patients consecutively enrolled in a nationwide cohort, including the majority of Hepatology Italian centers, clearly shows that clinical response frequently fluctuates. Indeed, the clinical manifestation pattern may change and reappear, either persistently or transiently, and this implies an attentive approach to post-SVR monitoring of CV patients, especially when they show symptom maintenance or recurrence. In this light, the accurate evaluation of both clinical and laboratory factors that represent prognostic indexes will aid in predicting different clinical evolutions. This could permit to tailor the frequency and quality of follow-up appointments, as recommended for HCV-related

liver damage, which will assist in the selection of the best therapeutic approach following HCV eradication.

Author Contributions

Conceptualization: LAK, ALZ. Data curation: VP, MGQ. Formal analysis: VP. Resources: MMo, GB, CM, MP, MMasa, IG, PA, SM, EB, RF, MP, ALF, DL, DI, CC, MGR, AB, GV, BC, CL, AI, AC, FPR, FB, FM, LC, MMass, PB, ALZ. Writing-original draft: LAK, ALZ, LG. Writing-review & editing: LAK, ALZ, LG, MGQ. Supervision: LAK, ALZ. All the authors have read and approved the final manuscript.

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Figure legends

Figure 1. Flow chart of evaluated patients in each sub analysis of clinical response following the Sustained Virological Response in patients with Hepatitis C virus related Cryoglobulinemic Vasculitis.

Figure 2. (A) Curve describing the first time in which a clinical response was observed (either Complete response, Full Complete Response or Partial Response) after end of Direct Acting Antiviral treatment in patients with Cryoglobulinemic Vasculitis. (B) Curve of Cryoglobulinemic Vasculitis relapse occurring during the follow-up after the first clinical response following Hepatitis C virus eradication.

Figure 3. Comparison between cryocrit, rheumatoid factor and C4 complement values before and after Direct Acting Antiviral treatment at different time points. Estimated means by mixed model (cryocrit n=144, RF n=42, C4 n=22) with at least a 1-year follow-up, cryocrit or rheumatoid factor or C4 complement data at the end of treatment and at least one other value during the follow-up (p value compared with baseline adjusted by Dunnet correction).

Figure 4. Pretreatment rheumatoid factor values in Cryoglobulinemic Vasculitis patients without (0) or with clinical relapse (1) following the Sustained Virological Response after Direct Acting Antiviral therapy.

References

1. Ramos-Casals M, Stone JH, Cid MC, Bosch X. The cryoglobulinaemias. Lancet 2012;379:348-360.
2. Ferri C, Ramos-Casals M, Zignego AL, Arcaini L, Roccatello D, Antonelli A, Saadoun D, et al. International diagnostic guidelines for patients with HCV-related extrahepatic manifestations. A multidisciplinary expert statement. Autoimmun Rev 2016;15:1145-1160.
3. Zignego AL, Ramos-Casals M, Ferri C, Saadoun D, Arcaini L, Roccatello D, Antonelli A, et al. International therapeutic guidelines for patients with HCV-related extrahepatic disorders. A multidisciplinary expert statement. Autoimmun Rev 2017;16:523-541.
4. Ferri C, Caracciolo F, Zignego AL, La Civita L, Monti M, Longombardo G, Lombardini F, et al. Hepatitis C virus infection in patients with non-Hodgkin's lymphoma. Br J Haematol 1994;88:392-394.
5. Mele A, Pulsoni A, Bianco E, Musto P, Szklo A, Sanpaolo MG, Iannitto E, et al. Hepatitis C virus and B-cell non-Hodgkin lymphomas: an Italian multicenter case-control study. Blood 2003;102:996-999.
6. Zignego AL, Ferri C, Giannini C, La Civita L, Careccia G, Longombardo G, Bellesi G, et al. Hepatitis C virus infection in mixed cryoglobulinemia and B-cell non-Hodgkin's lymphoma: evidence for a pathogenetic role. Arch Virol 1997;142:545-555.
7. Meltzer M, Franklin EC, Elias K, McCluskey RT, Cooper N. Cryoglobulinemia--a clinical and laboratory study. II. Cryoglobulins with rheumatoid factor activity. Am J Med 1966;40:837-856.
8. Desbois AC, Cacoub P, Saadoun D. Cryoglobulinemia: An update in 2019. Joint Bone Spine 2019;86:707-713.
9. Gulli F, Basile U, Gragnani L, Napodano C, Pocino K, Miele L, Santini SA, et al. IgG cryoglobulinemia. Eur Rev Med Pharmacol Sci 2018;22:6057-6062.
10. De Vita S, Soldano F, Isola M, Monti G, Gabrielli A, Tzioufas A, Ferri C, et al. Preliminary classification criteria for the cryoglobulinaemic vasculitis. Ann Rheum Dis 2011;70:1183-1190.
11. Cacoub P, Saadoun D. Extrahepatic Manifestations of Chronic HCV Infection. N Engl J Med 2021;384:1038-1052.
12. Roccatello D, Saadoun D, Ramos-Casals M, Tzioufas AG, Fervenza FC, Cacoub P, Zignego AL, et al. Cryoglobulinaemia. Nat Rev Dis Primers 2018;4:11.
13. Zignego AL, Giannini C, Gragnani L. HCV and lymphoproliferation. Clin Dev Immunol 2012;2012:980942.
14. Boleto G, Ghillani-Dalbin P, Musset L, Biard L, Mulier G, Cacoub P, Saadoun D. Cryoglobulinemia after the era of chronic hepatitis C infection. Semin Arthritis Rheum 2020;50:695-700.

15. Pietrogrande M, De Vita S, Zignego AL, Pioltelli P, Sansonno D, Sollima S, Atzeni F, et al. Recommendations for the management of mixed cryoglobulinemia syndrome in hepatitis C virus-infected patients. Autoimmun Rev 2011;10:444-454.

16. La Civita L, Zignego AL, Lombardini F, Monti M, Longombardo G, Pasero G, Ferri C. Exacerbation of peripheral neuropathy during alpha-interferon therapy in a patient with mixed cryoglobulinemia and hepatitis B virus infection. J Rheumatol 1996;23:1641-1643.

17. Gragnani L, Visentini M, Fognani E, Urraro T, De Santis A, Petraccia L, Perez M, et al. Prospective study of guideline-tailored therapy with direct-acting antivirals for hepatitis C virus-associated mixed cryoglobulinemia. Hepatology 2016;64:1473-1482.

18. Gragnani L, Cerretelli G, Lorini S, Steidl C, Giovannelli A, Monti M, Petraccia L, et al. Interferon-free therapy in hepatitis C virus mixed cryoglobulinaemia: a prospective, controlled, clinical and quality of life analysis. Aliment Pharmacol Ther 2018;48:440-450.

19. Cacoub P, Si Ahmed SN, Ferfar Y, Pol S, Thabut D, Hezode C, Alric L, 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.

20. Saadoun D, Thibault V, Si Ahmed SN, Alric L, Mallet M, Guillaud C, Izzedine H, et al. Sofosbuvir plus ribavirin for hepatitis C virus-associated cryoglobulinaemia vasculitis: VASCUVALDIC study. Ann Rheum Dis 2016;75:1777-1782.

21. Passerini M, Schiavini M, Magni CF, Landonio S, Niero F, Passerini S, Croci AL, et al. Are direct-acting antivirals safe and effective in hepatitis C virus-cryoglobulinemia? virological, immunological, and clinical data from a real-life experience. Eur J Gastroenterol Hepatol 2018;30:1208-1215.

22. Bonacci M, Lens S, Mariño Z, Londoño MC, Rodriguez-Tajes S, Sánchez-Tapias JM, Ramos-Casals M, et al. Long-Term Outcomes of Patients With HCV-Associated Cryoglobulinemic Vasculitis After Virologic Cure. Gastroenterology 2018;155:311-315.e316.

23. Sollima S, Milazzo L, Peri AM, Torre A, Antinori S, Galli M. Persistent mixed cryoglobulinaemia vasculitis despite hepatitis C virus eradication after interferon-free antiviral therapy. Rheumatology (Oxford) 2016;55:2084-2085.

24. Sollima S, Milazzo L, Vassalini P, Antinori S, Galli M. Recurrence of mixed cryoglobulinaemia vasculitis following influenza vaccination despite clearance of hepatitis C virus infection. Clin Exp Rheumatol 2018;36 Suppl 111:161-162.

25. Emery JS, Kuczynski M, La D, Almarzooqi S, Kowgier M, Shah H, Wong D, et al. Efficacy and Safety of Direct Acting Antivirals for the Treatment of Mixed Cryoglobulinemia. Am J Gastroenterol 2017;112:1298-1308.

26. Visentini M, Quartuccio L, Del Padre M, Colantuono S, Minafò YA, Fiorilli M, De Vita S, et al. Late relapses of hepatitis C virus-cured mixed cryoglobulinaemia associated with infection or cancer. Rheumatology (Oxford) 2018;57:1870-1871.
27. Gragnani L, Lorini S, Marri S, Basile U, Santarlasci V, Monti M, Madia F, et al. Hematological and Genetic Markers in the Rational Approach to Patients With HCV Sustained Virological Response With or Without Persisting Cryoglobulinemic Vasculitis. Hepatology 2021.
28. Kondili LA, Vella S, Group PC. PITER: An ongoing nationwide study on the real-life impact of direct acting antiviral based treatment for chronic hepatitis C in Italy. Dig Liver Dis 2015;47:741-743.
29. Quartuccio L, Isola M, Corazza L, Ramos-Casals M, Retamozo S, Ragab GM, Zoheir MN, et al. Validation of the classification criteria for cryoglobulinaemic vasculitis. Rheumatology (Oxford) 2014;53:2209-2213.
30. Stasi C, Triboli E, Arena U, Urraro T, Petrarca A, Gragnani L, Laffi G, et al. Assessment of liver stiffness in patients with HCV and mixed cryoglobulinemia undergoing rituximab treatment. J Transl Med 2014;12:21.
31. Castéra L, Le Bail B, Roudot-Thoraval F, Bernard PH, Foucher J, Merrouche W, Couzigou P, et al. Early detection in routine clinical practice of cirrhosis and oesophageal varices in chronic hepatitis C: comparison of transient elastography (FibroScan) with standard laboratory tests and non-invasive scores. J Hepatol 2009;50:59-68.
32. Kondili LA, Vella S, Zignego AL, Group Pc. Mixed cryoglobulinaemia: An important but frequently unrecognized and underestimated HCV-related condition in the real life practice. Liver Int 2018;38:183.
33. Gragnani L, Fognani E, Piluso A, Boldrini B, Urraro T, Fabbrizzi A, Stasi C, 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.
34. Galli M, Oreni L, Saccardo F, Castelnovo L, Filippini D, Marson P, Mascia MT, et al. HCV-unrelated cryoglobulinaemic vasculitis: the results of a prospective observational study by the Italian Group for the Study of Cryoglobulinaemias (GISC). Clin Exp Rheumatol 2017;35 Suppl 103:67-76.
35. Kayali Z, Buckwold VE, Zimmerman B, Schmidt WN. Hepatitis C, cryoglobulinemia, and cirrhosis: a meta-analysis. Hepatology 2002;36:978-985.
36. Saadoun D, Pol S, Ferfar Y, Alric L, Hezode C, Si Ahmed SN, de Saint Martin L, et al. Efficacy and Safety of Sofosbuvir Plus Daclatasvir for Treatment of HCV-Associated Cryoglobulinemia Vasculitis. Gastroenterology 2017;153:49-52.e45.
37. Kondili LA, Gaeta GB, Brunetto MR, Di Leo A, Iannone A, Santantonio TA, Giammario A, et al. Incidence of DAA failure and the clinical impact of retreatment in real-life patients treated in the

advanced stage of liver disease: Interim evaluations from the PITER network. PLoS One 2017;12:e0185728.

38. Bonacci M, Lens S, Londoño MC, Mariño Z, Cid MC, Ramos-Casals M, Sánchez-Tapias JM, 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.e571.

39. Lauletta G, Russi S, Pavone F, Vacca A, Dammacco F. Direct-acting antiviral agents in the therapy of hepatitis C virus-related mixed cryoglobulinaemia: a single-centre experience. Arthritis Res Ther 2017;19:74.

40. Pozzato G, Mazzaro C, Artemova M, Abdurakhmanov D, Grassi G, Crosato I, Mauro E, et al. Direct-acting antiviral agents for hepatitis C virus-mixed cryoglobulinaemia: dissociated virological and haematological responses. Br J Haematol 2020.

41. Artemova M, Abdurakhmanov D, Ignatova T, Mukhin N. Persistent hepatitis C virus-associated cryoglobulinemic vasculitis following virus eradication after direct-acting antiviral therapy. Hepatology 2017;65:1770-1771.

42. Gragnani L, Lorini S, Zignego AL. Reply: "Recurrent cryoglobulinemic vasculitis in the era of direct acting antivirals: A story beyond SVR 12.". Hepatology 2021.

43. Visentini M, Del Padre M, Colantuono S, Yang B, Minafò YA, Antonini S, Carnovale M, 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.

44. Gulli F, Santini SA, Napodano C, Bottoni P, Pocino K, Rapaccini GL, Basile U. Cryoglobulin Test and Cryoglobulinemia Hepatitis C-Virus Related. Mediterr J Hematol Infect Dis 2017;9:e2017007.

Please note that author names in bold designate shared co-first authorship.

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Table 1. Demographic and clinical characteristics of patients with HCV related Mixed Crioglobulinemia who achieved a SVR

	Patients with Cryoglobulinemic Vasculitis (CV) N=423*	**Patients with Asymptomatic Mixed Cryoglobulinemia (MC) N=655**	**P value**
Age (years) (mean, sd)	62.7 ± 12.1	62.2 ± 12.6	0.444
Sex (%, no./Pts)			
Male	35.9% (152/ 423)	47.3% (310/ 655)	<0.001
Female	64.1% (271/ 423)	52.7% (345/ 655)	
Fibrosis distribution *(%, no./Pts)*			0.013
F0-F1	42.7% (167/ 390)	33.9% (204/ 602)	
F2	10.5% (41/ 390)	9.1% (55/ 602)	
F3	9.2% (36/ 390)	9.8% (59/ 602)	
F4-Cirrhosis	37.4% (146/ 390)	47.2% (284/ 602)	

*In patients with Cryoglobulinemic Vasculitis who achieved a SVR, clinical data regarding the cryoglobulinemic syndrome were missing for 143 patients

Table 2. Factors associated with clinical response in patients with HCV related Cryoglobulinemic Vasculitis at the end of the antiviral treatment - Univariate and multivariate analysis

		Univariate analysis* N=309		Multivariate analysis N=309	
		OR (95% CI)	**P value**	**OR (95% CI)**	**P value**
Age (years)		1.02 (1.00-1.04)	0.052	1.02 (1.00-1.04)	0.039
Sex	Male	1			
	Female	0.90 (0.56-1.43)	0.646		
Purpura	No	1			
	Yes	1.01 (0.59-1.75)	0.964		
Asthenia	No	1			
	Yes	1.33 (0.77-2.30)	0.308		
Arthralgia	No	1			
	Yes	1.05 (0.64-1.70)	0.853		
Neuropathy	No	1			
	Yes	1.15 (0.73-1.81)	0.557		
Renal involvement	No	1			
	Yes	1.70 (0.92-3.16)	0.093	1.79 (0.96-3.36)	0.058
Xerostomia/Xerophthalmia	No	1			
	Yes	1.27 (0.80-2.01)	0.308		
Raynaud	No	1			
	Yes	0.93 (0.53-1.65)	0.811		
Ulcer	No	1			
	Yes	0.88 (0.24-3.18)	0.843		
Pretreatment Cryocriot		1.01 (0.96-1.06)	0.68		
Pretreatment Rheumatoid Factor		1.00 (1.00-1.00)	0.559		
Pretreatment C4		1.03 (0.88-1.21)	0.694		
Rituximab	Yes				
	No	1.65 (0.69-3.93)	0.262		

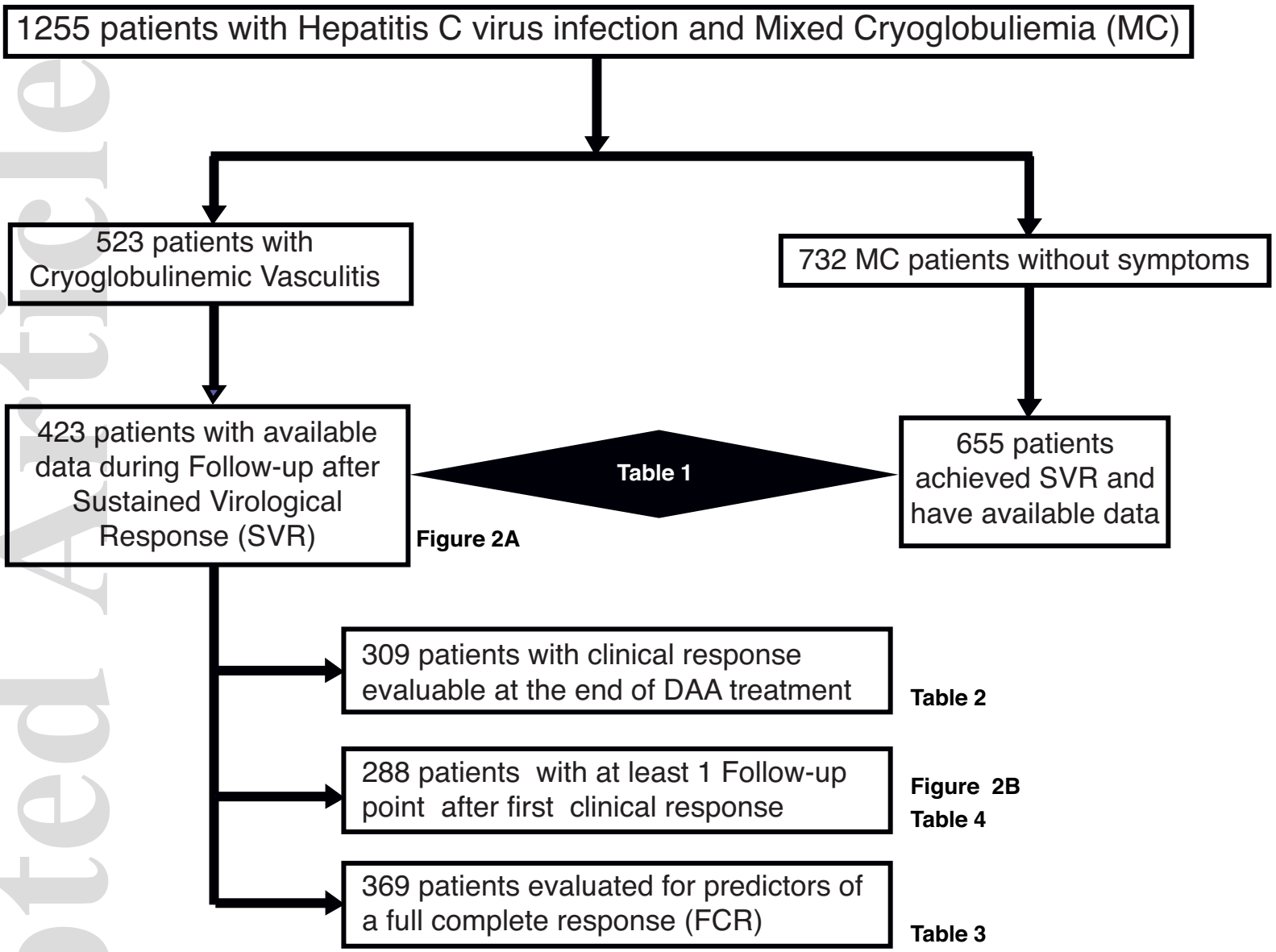
*Out of 423 patients with Cryoglobulinemic Vasculitis, 309 were evaluated for the clinical response at the end of the follow up, the remaining were evaluated in different follow-up time points.

Table 3. Pre-treatment factors associated with a Full Complete Response without clinical deterioration or relapse in patients with HCV related Cryoglobulinemic Vasculitis – Cox regression analysis

		Univariate analysis N=369		Multivariate analysis N=278	
Variable		**HR (CI 95%)**	**p value**	**HR (CI 95%)**	**p value**
Age (years)		0.96 (0.94-0.98)	<.0001	0.96 (0.94-0.99)	0.002
Sex	Male	1			
	Female	0.42 (0.25-0.69)	0.001		
Purpura	No	1			
	Yes	0.32 (0.14-0.74)	0.008		
Asthenia	No	1			
	Yes	0.41 (0.25-0.68)	0.001	0.53 (0.26-1.10)	0.088
Arthralgia	No	1			
	Yes	0.44 (0.27-0.72)	0.001		
Neuropathy	No	1			
	Yes	0.4 (0.23-0.69)	0.001	0.4 (0.18-0.87)	0.022
Renal involvement	No	1			
	Yes	0.75 (0.37-1.53)	0.434		
Xerostomia/Xerophthalmia	No	1			
	Yes	0.6 (0.36-1.00)	0.051		
Raynaud	No	1			
	Yes	0.54 (0.25-1.19)	0.128		
Ulcers		1.04 (0.25-4.24)	0.960		
Pretreatment Cryocrit		0.81 (0.67-0.99)	0.041	0.81 (0.66-0.98)	0.03
Pretreatment Rheumatoid Factor		1 (0.99-1)	0.202		
Pretreatment C4		1.2 (0.97-1.48)	0.090		

Table 4. Pretreatment factors associated with Relapse after clinical response in patients with HCV related Cryoglobulinemic Vasculitis - Cox regression analysis

		Univariate analysis N=288		Multivariate analysis N=94	
Variable		**HR (CI 95%)**	**p value**	**HR (CI 95%)**	**p value**
Age (years)		1.01 (1.00-1.02)	0.178		
Sex	Male	1			
	Female	1.01 (0.71-1.44)	0.937		
Purpura	No	1			
	Yes	0.67 (0.45-1.01)	0.055	0.75 (0.41-1.37)	0.349
Asthenia	No	1			
	Yes	1.08 (0.71-1.64)	0.730		
Arthralgia	No	1			
	Yes	0.89 (0.63-1.26)	0.507		
Neuropathy	No	1			
	Yes	1.34 (0.95-1.88)	0.092	1.38 (0.74-2.56)	0.313
Renal involvement	No	1			
	Yes	0.91 (0.58-1.41)	0.672		
Xerostomia Xerophthalmia	No	1			
	Yes	1.41 (1.01-1.99)	0.047	0.84 (0.52-1.70)	0.841
Raynaud	No	1			
	Yes	0.87 (0.57-1.32)	0.512		
Ulcer	No	1			
	Yes	0.43 (0.11-1.72)	0.232		
Pretreatment Cryocrit		0.99 (0.94-1.03)	0.514		
Pretreatment Rheumatoid Factor		1 (1.00-1.001)	0.017	1 (1.00-1.001)	0.021
Pretreatment C4		0.99 (0.89-1.09)	0.786		
Rituximab	No	1			
	Yes	0.65 (0.29-1.48)	0.303		



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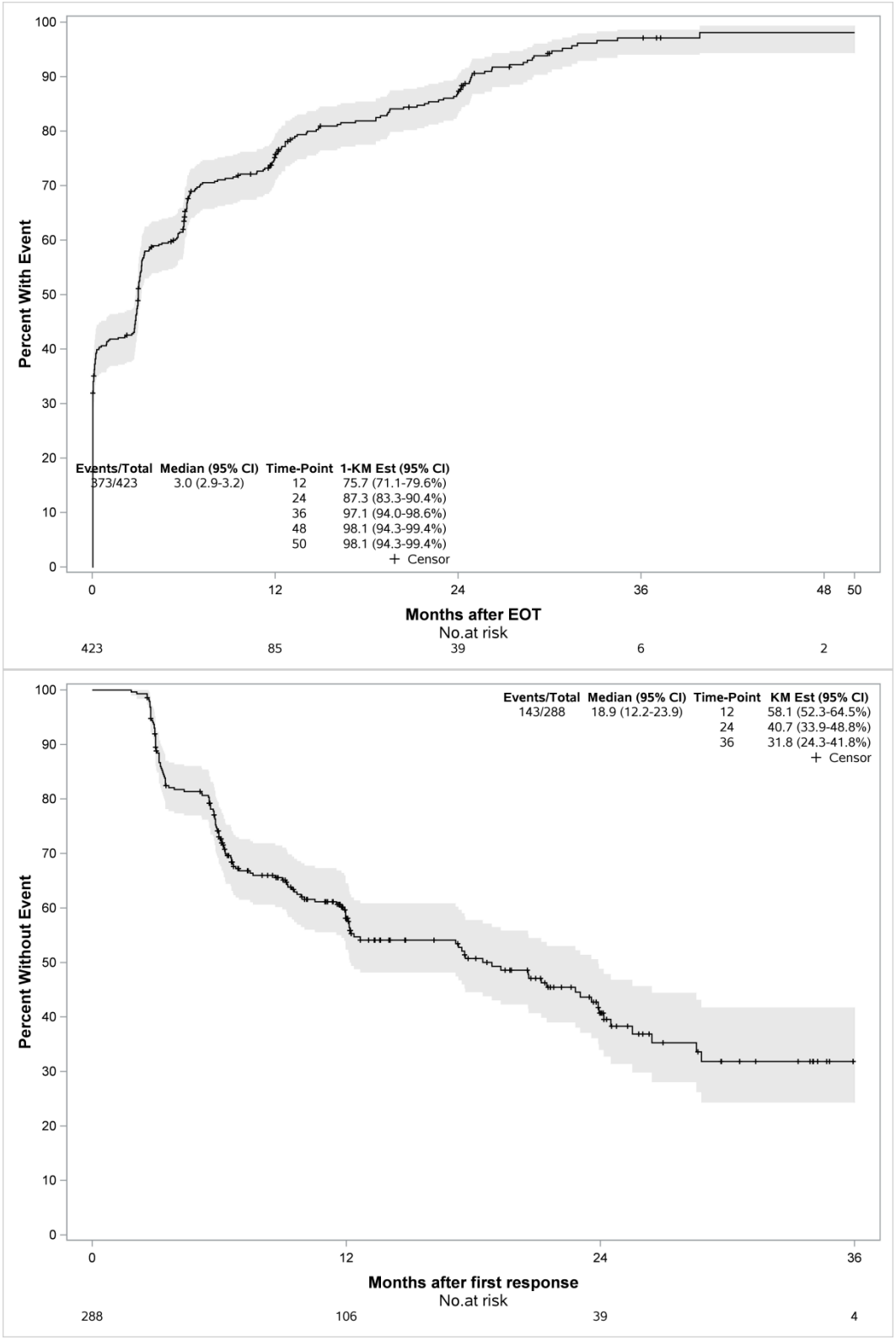

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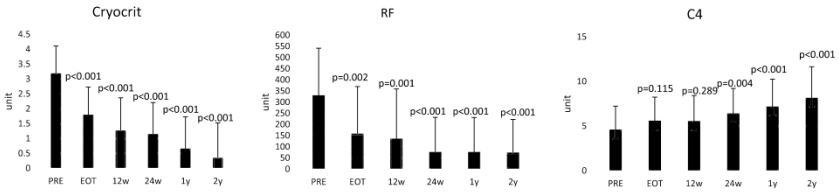

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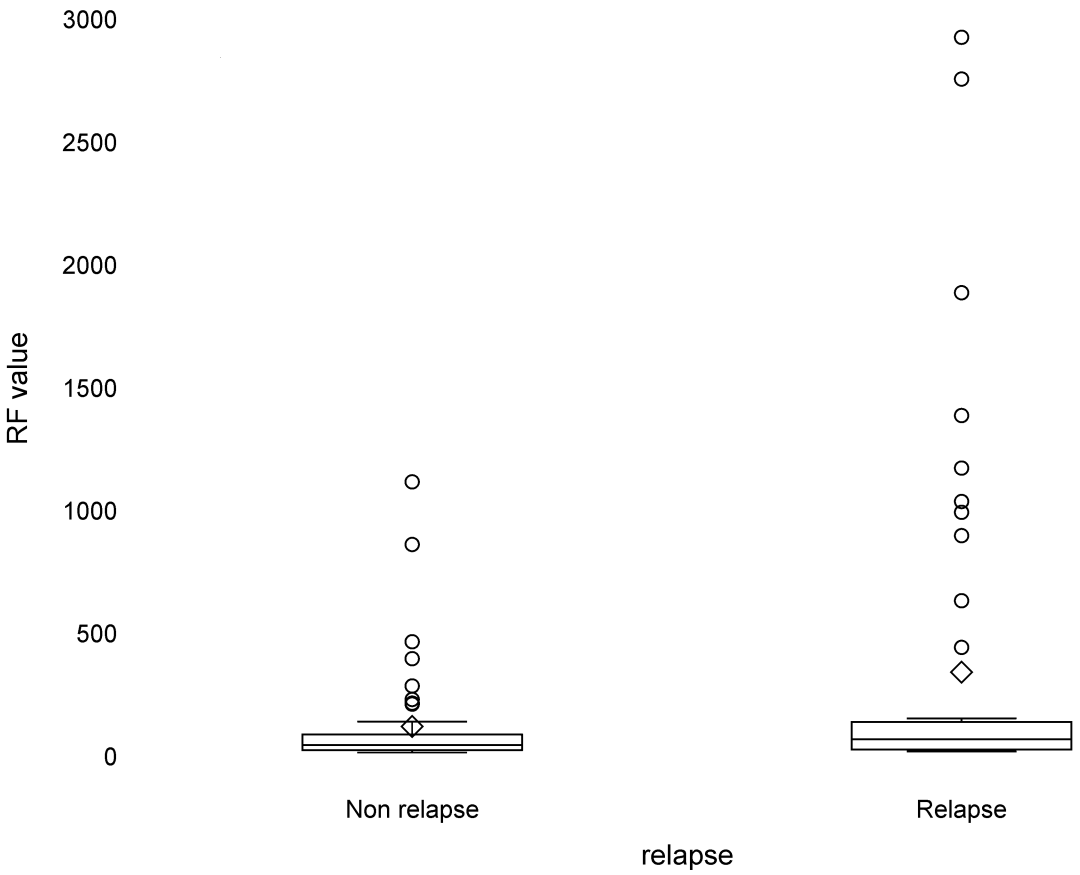

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