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This is the author's manuscript

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/1715039> since 2020-01-21T15:56:52Z

Published version:

DOI:10.1097/BOR.0000000000000624

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This is the author's final version of the contribution published as:

Curr Opin Rheumatol. 2019 Sep;31(5):499-504. doi: 10.1097/BOR.0000000000000624.

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The publisher's version is available at:

Curr Opin Rheumatol. 2019 Sep;31(5):499-504. doi:

10.1097/BOR.0000000000000624. The dilemma of treating hepatitis C virus-associated cryoglobulinemia. Roccatello D, Fenoglio R, Sciascia S.

When citing, please refer to the published version.

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The dilemma of treating hepatitis C virus-associated cryoglobulinemia

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Abstract

Purpose of review: the present review focuses on the new therapeutic opportunities offered by the combination of biological drugs, mainly Rituximab, with Direct-acting Antiviral Agents (DAAs).

Recent findings: hepatitis C virus (HCV) is known to be the etiologic agent in the majority of patients with mixed cryoglobulinemia syndrome (MC). Clinical research has been focused on anti-viral drugs and, more recently, on the new, highly potent DAAs. New DAAs assure sustained virologic response (SVR) rates >90% with relief of mild to moderate symptoms.

Summary: MC may present with multi-organ vasculitis involving kidneys, joints, skin, and peripheral nerves. Data on DAAs efficacy in HCV-associated cryoglobulinemic vasculitis (CV) are disappointing possibly due to the inability of these drugs to suppress the immune-mediated process once it has been triggered. Immunosuppression has often been employed in the past as a first-line therapy in CV despite the potential risk of the infection exacerbation. However, more manageable Rituximab-based therapeutic approaches have been more recently used without increase of viral load. Rituximab substantially changed the outcome of HCV-associated cryoglobulinemic vasculitis by providing long-term remission. A combination schedule of DAAs and Rituximab may result in eradication of both CV and HCV infection.

Keywords: mixed cryoglobulinemia syndrome, cryoglobulinemic vasculitis, Rituximab, Direct-acting Antiviral Agents.

Abbreviations: Hepatitis C Virus (HCV), cryoglobulinemia syndrome (CM), cryoglobulinemic vasculitis (CV), immune complexes (ICs), pegylated-interferon- α (peg-IFN α), ribavirin (RBV), nonstructural (NS), direct-acting antiviral agents (DAAs).

Introduction

Mixed cryoglobulinemia syndrome is characterised by the appearance in the circulation of mixed cryoglobulins, a particular type of immune complexes (ICs) that may deposit in several tissues. Several conditions can be found to be associated with MC, but most of them are related to a HCV infection [1-6]. While circulating cryoglobulins may be detected in about 40% of infected patients and are usually asymptomatic, a CV occurs in a minority of cases (<2%) [7] with long-term HCV infection and older age [8,9].

Purpose of the present review is to provide data on the therapeutic opportunities of CV offered by the DAAs and biological drugs, mainly Rituximab.

Pathogenic aspects relevant to therapy

HCV infects not only hepatocytes, but also B-lymphocytes, macrophages, peripheral dendritic cells and monocytes [10]. More specifically, HCV-RNA, HCV-NS3 and core proteins have been restrictively found in CD19-positive B-cells [11], which incidentally are the target cells for Rituximab-based regimens. HCV stimulation enhances the expression of lymphomagenesis-related genes [12]. Under this trigger effect, permanent clones of B-lymphocytes produce oligo- or monoclonal IgM that are known to display rheumatoid factor activity, favouring the formation of ICs formed by the monoclonal IgM itself, HCV, and anti-HCV polyclonal IgG antibodies. Due to the presence of IgM, these ICs escape the splenic and hepatic macrophage removal system [13], and, when phagocytosed, are not degraded by monocytes as shown both in humans [14] and in murine models [15]. The release of pro-inflammatory cytokines and

pro-cathepsin D from activated macrophages perpetuates tissue damage [15]. Based on these pathogenic principles it seems unlikely that antiviral agents can effectively interfere with the pathogenesis of MC vasculitis once the immune process is definitively triggered, and impact the immune-mediated injury.

Rituximab as a target therapeutic approach for CV

Rituximab is a monoclonal antibody directed at the lymphocyte membrane protein CD20. Rituximab selectively depletes the CD20/CD19+veB lymphocytes, thereby abolishing IgM production and new cryoglobulin formation. It is noteworthy that HCV proteins and RNA have been restrictively detected in these cells [11].

Several hundred of Rituximab-treated patients have been reported in the literature [16–37]. This agent has been proven to be effective in the management of skin ulcers, renal manifestations in 75–90% of case, and sensitive-motor neuropathy in 70%. Rituximab depletes bone marrow B-cell clonal expansion resulting in a decrease of serum cryoglobulins and rheumatoid factor with normalisation of C4 levels [35–39].

We prospectively evaluated the very long-term effects (mean follow-up 72.47 months) of Rituximab in patients with severe CV [35]. Rituximab was given to 31 patients (27 HCV+ve) with MC (type II in 29 subjects and type III in 2) and diffuse membranoproliferative glomerulonephritis (16 cases), sensitive-motor neuropathy (26 cases) and severe skin ulcers (7 cases). Rituximab was given at a dose of 375 mg/m² (days 1, 8, 15 and 22, followed by 2 more doses 1 and 2 months later, the so-called “4 plus 2 protocol”). Five patients also received 3 pulses of 500 mg of methylprednisolone. No other immunosuppressive or antiviral drugs were added. A complete remission of pre-treatment active manifestations in all cases of purpuric lesions and non-healing vasculitic ulcers (Figure 1), and in 80% of the peripheral neuropathies was achieved. Re-induction with Rituximab was given in 9 relapsing patients after a mean of 31.1 months, resulting again effective. After 6 years of follow-up, the survival

rate was 75% and the probability of remaining symptom-free for 10 years without any therapy was 60% after a single “4 plus 2” cycle, while the likelihood of living symptom-free for 5 years after relapse was 80% if treated with the same protocol. Viral load did not increase in HCV+ve patients. This open study showed Rituximab to be safe and effective for treating the most severe cases of MC, even in a very long-term perspective (6 years).

16 open-label trials with at least 10 patients with 440 patients could be recruited [16-31]. Median complete remission (CR) was 68%, partial remission (PR) was 14%, and no response was 10%.

Focusing on case series reporting renal outcomes [16-18, 21, 23-27, 30, 31], 143 patients could be identified, but only a minority had biopsy-proven evidence of cryoglobulinemic glomerulonephritis. Median rates of renal complete and partial remission were 57% and 20%, respectively.

Among the three RCTs [32-34], in 2010 Dammacco and co-workers [32] investigated the safety and efficacy of a combined use of pegylated-interferon- α (peg-IFN α) and ribavirin (RBV), with or without Rituximab, in HCV-related MC. Twenty-two patients with HCV-related MC received peg-IFN α weekly plus RBV for 48 weeks plus Rituximab 375 mg/m² once a week for 1 month followed by two 5-monthly infusion. Fifteen additional patients received peg-IFN α and RBV with the same modalities, but without Rituximab. In Rituximab group CR was achieved in 54.5% (versus 33.3% of the control group, $p < 0.05$). In a prospective multi-center RCT investigating Rituximab in severe CV, 59 patients were randomized to receive a conventional treatment (consisting of one of the following 3 options: glucocorticoids; azathioprine or cyclophosphamide; plasmapheresis) or Rituximab (2 infusions of 1 g each)[34]. Rituximab resulted to be the best therapy for all 3 target-organ manifestations (skin ulcers, active glomerulonephritis, or refractory peripheral neuropathy). The number of patients who achieved the primary endpoint was statistically higher in the Rituximab group both at 1 and 2 years ($p < 0.0001$).

In a single-center, open label, RCT, Rituximab was compared to the best available therapy in patients affected by HCV-associated CV. Six months after the beginning of the treatment 10 out of 12 patients in

the Rituximab group (83%) and 1 of 12 patients in the control group (8%) were in remission ($p < 0.001$) [33]. No effects of Rituximab on HCV viremia was observed.

Notably, by examining results from 11 studies using Rituximab (administered either in 4 weekly infusions or as 1 g given 15 days apart) in which renal outcome was addressed, the median CR rate of 57%. In a large cohort of renal patients given the 4+2 regimen CR was achieved in 75% of cases. The “4 plus 2 protocol might intensify the depleting effect of Rituximab on lymphocytes when compared to other Rituximab regimens (i.e., “Lymphoma” and “Rheumatoid Arthritis” protocols), thus maintaining a more prolonged B-cell depletion and improving clinical outcome.

MC is an immune-mediated process that becomes independent from the triggering virus. Rituximab is much more selective than conventional immunosuppressive treatments at interfering with the downstream processes following the disease trigger [35-36], and is definitely safer, as shown in Figure 2. No increase in viraemia could be detected possibly due to Rituximab depleting effects of the CD19+ve cells which serve as reservoir for HCV. Rituximab specifically targets the nephrotoxic Ig-producing cells inducing a lymphocyte subpopulation re-assessment (Figure 3).

The potential role of direct antiviral agents

Before the advent of direct-acting antiviral agents (DAAs), chronic HCV infection was mainly treated with peg-IFN and RBV [37] which achieved eradication of infection in fewer than 50% of naive patients with genotype 1 infection [38,39].

With regard to HCV-associated CV, PegIFN/RBV therapy was found to be consistently effective only when given with Rituximab either in a combined [32] or sequential [21] scheme.

The new DAAs showed significant antiviral efficacy (>90% cure) and a good tolerance profile. The new drugs target the 3 nonstructural proteins: NS5A and NS5B RNA polymerase, the NS3 serine protease and its cofactor, NS4A. Daclatasvir was the first NS5A inhibitor to be launched, [42], followed by ledipasvir[43] and ombitasvir[37]. Overall, these DAAs are effective against a wide spectrum of HCV

genotypes [41]. To date, sofosbuvir is the most advanced DAAs. Initially approved to be used in association with RBV for HCV infection (genotypes 2 and 3), it was the first all-oral, PegIFN-free regimen. Multidrug regimens are combinations of an NS3/4A inhibitor, an NS5A inhibitor and a non-nucleoside NS5A inhibitor [44-46]. A full 3D treatment regimen achieves an SVR rate of >95% when administered for 12 weeks to naive patients, and >90% in prior non-responders.

DAAs could be expected to modify the incidence of vasculitis being the result of a prolonged history of HCV infection. However, these agents do not possess the immunomodulatory effects of the interferons. They likely do not interfere with the pathogenesis of MC vasculitis nor effectively impact on the development of the immune-mediated injury once the immune disorder is established. Besides, the uncertainty of their pharmacokinetics and safety in the case of renal impairment implies caution in nephritic patients.

Results of the DAAs regimens in patients with HCV-related CV are difficult to interpret. Makara [40] reported remission of multiorgan involvement and withdrawal of serum cryoglobulins 4 weeks after initiating DAA-therapy in a severe case of HCV-associated CV. But this patient also had received 1,600 mg of Rituximab in four doses 5 months earlier. Sise et al. [47] showed that patients with CV had sustained virological response rate at 12 weeks of 83% (10 out of 12 patients) with a sofosbuvir-based DAA regimen. Patients with glomerulonephritis (7 out of 12) showed a reduction in proteinuria and eGFR improvement. But only 2 patients had a substantial amelioration of serum creatinine (one concomitantly treated with Rituximab and one whose renal involvement was not documented by renal biopsy). Functional changes were negligible in 4 patients, while serum creatinine increased over time in the remaining patient. As regard to the urinary abnormalities, the only patient who had nephrotic range proteinuria at baseline (dropped to non-nephrotic values) received Rituximab together with DAAs. Another patient who showed a moderate reduction of proteinuria (from 1,574 to 800 mg/g sCr) was concomitantly treated with ustekinumab, an anti-IL-12-23 monoclonal antibody, whose effects in proteinuric patients is presently unpredictable. Proteinuria decreased from 2,141 to 400 mg/g sCr in a

patient who had not undergone biopsy. Of the remaining 4 patients, proteinuria was negative in one case, not determined in another, and only determined by urinalysis in 2 cases.

Sollima et al [41] treated 7 patients with HCV-associated CV with new DAAs within an expanded access program. Nephropathy was the most frequent manifestation, presenting as a severe nephrotic syndrome, stages III-IV chronic kidney disease or both. Patients were treated with a variety of DAA regimens, including ombitasvir/paritaprevir/ritonavir and dasabuvir, sofosbuvir plus ribavirin, sofosbuvir plus daclatasvir and sofosbuvir plus simeprevir, depending on HCV genotypes. Treatment was given for 12 weeks in five cases and 24 weeks in two. All patients achieved SVR at post-treatment week 12 (confirmed at week 24 in five of them, on a longer follow-up). Serum cryoglobulins were undetectable in 4 patients at the end of treatment but increased again in three during follow-up. At post-treatment week 12, clinical response was observed only in 2 patients. Specifically, the response was partial in one patient and complete in another who experienced vasculitis relapse despite still undetectable HCV RNA. Cornella and coworkers [48] reported 5 patients with genotype 1 chronic HCV-related hepatitis complicated by MC who received 24 weeks triple therapy with boceprevir, telaprevir and sofosbuvir. Clearance of serum cryoglobulins was not achieved in any of these patients.

Lauletta et al. [49] described 22 patients with HCV-associated CV treated with new DAAs. Despite a sustained virological response in 63.7%, only partial benefits were observed in patients with peripheral neuropathy. The Authors inferred that the pathogenic process underlying CV might have progressed despite viral clearance and that a “point of no return” might have been overstepped. In a prospective study on HCV pts with symptomatic or asymptomatic cryoglobulinemia receiving DAAs, 71% of patients with vasculitis achieved a complete clinical response, including all 7 patients with kidney involvement. Since vasculitis relapsed in some patients, the authors speculated that incomplete suppression of the B-cell clonal proliferation might underlie the risk of virus-independent relapses [50].

Emery et al., [51] retrospectively evaluated 18 HCV-CV before and after treatment with DAA + pegINF. High sustained virological response (16/18) did not directly translate into improved immunological outcomes (with only 29.4% of patients having complete cryoprecipitate clearance). Among the 7 patients with severe CV included in the study, only one had a complete clinical response, 3 showed a partial clinical response and 2 no improvement. The Authors concluded that patients with life-threatening vasculitis were not susceptible of immunological and clinical response. In a prospective international multicenter cohort study on 148 patients with symptomatic HCV- CV receiving DAAs followed-up for 15.3 months, DAAs therapy achieved high virological responses in most patients, with some immunological and clinical benefits but only partial hematological changes (and detectable cryoglobulins in half of the patients) [52]. Factors associated with no or partial response to therapy included severe form of CV and peripheral neuropathy [52].

Conclusion

DAAs therapy in HCV-associated CV was found to achieve high virological responses with some immunological, clinical and hematological changes. However, these observations emphasised the role of DAAs in eradicating HCV infection even in MC patients, a subset that had been found less responsive to conventional antiviral treatment with Peg-IFN/RBV.

General drawbacks of these studies included the lack of histological evidence of renal involvement in the vast majority of cases, what made uncertain the nature of the functional impairment or the urinary abnormalities in patients with co-morbidities potentially responsible for the renal manifestations. Moreover, Del Padre et al. (53) observed that the clonal B cell could persist after the withdrawal of HCV, but underwent functional changes that might decrease their capacity to secrete pathogenic antibody. Events able to perturb immunologic homeostasis could reactivate these cells and lead to the resurgence of CV. Finally, virus eradication does not necessarily mean

that the immunological process has been stopped and several patients continue to have B lymphocyte clonal expansion after SVR.

A complete remission of MC associated with sustained virological response following a combined regimen of Peg-IFN+RBV+DAA plus Rituximab was described by Urraro et al [42]. A combination of DAAs and Rituximab can be effective both in CV treatment and in HCV eradication. Rituximab, able to deplete CD19 positive-B cells, known to be HCV reservoirs, could contribute to viral eradication making its combination with DAAs particularly attractive in the treatment of HCV-related CV. However, the optimal combination protocol of Rituximab plus DAAs need to be defined (54). Severe CV, especially with renal involvement, should first be treated with a Rituximab-based protocol (55). DAAs may be administered concomitantly or may be delayed (especially in the absence of a high viral load) pending on individual circumstances.

Key points:

- Mixed cryoglobulinemia is an immune-mediated process that becomes independent from the triggering virus. Rituximab is much more selective than conventional immunosuppressive treatments at interfering with the downstream processes following the disease trigger.
- Data on DAAs efficacy in HCV-associated cryoglobulinemic vasculitis are disappointing possibly due to the inability of these drugs to suppress the immune-mediated process once it has been triggered.
- Rituximab, able to deplete CD19 positive-B cells, known to be HCV reservoirs, could contribute to viral eradication making its combination with DAAs particularly attractive in the treatment of HCV-related CV

Acknowledgements:

None

Financial support and sponsorship:

None

Conflicts of interest:

None



Fig. 1. Skin ulcers healing after Rituximab treatment.

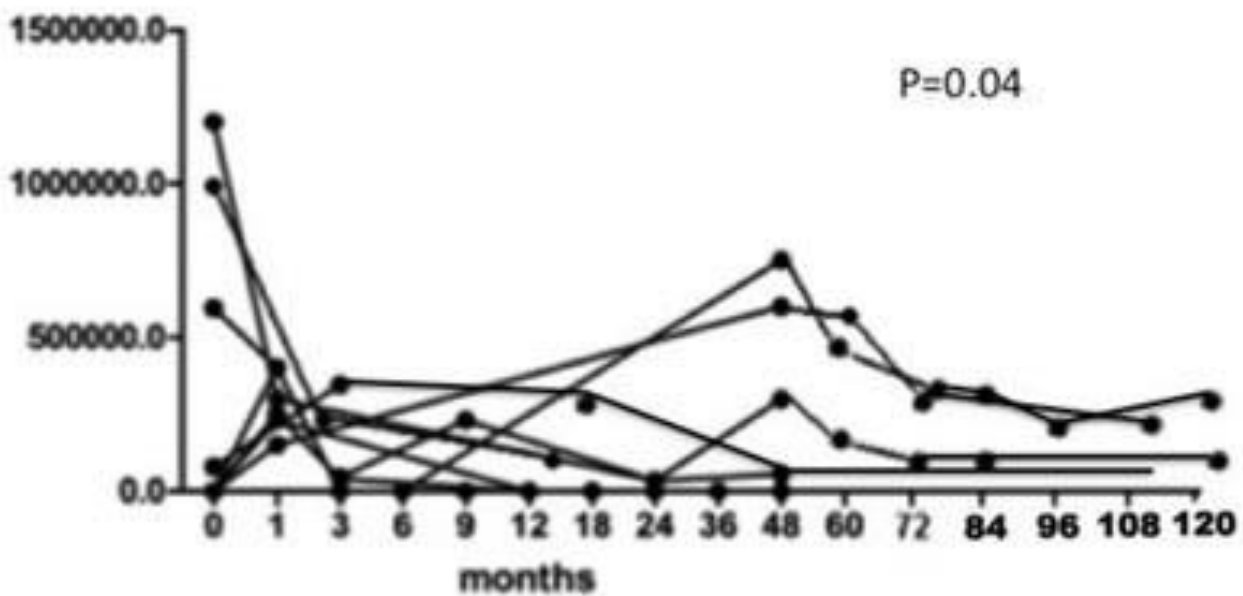


Fig 2. HCV RNA serum load as evaluated at 0, 3, 6, 9, 12 and 18 months and then yearly after Rituximab administration in our cohort of 31 patients with severe mixed cryoglobulinemic vasculitis (type II in 29 cases and type III in 2) with diffuse membranoproliferative glomerulonephritis (16 cases), peripheral neuropathy (#26) and large skin ulcers (#7)

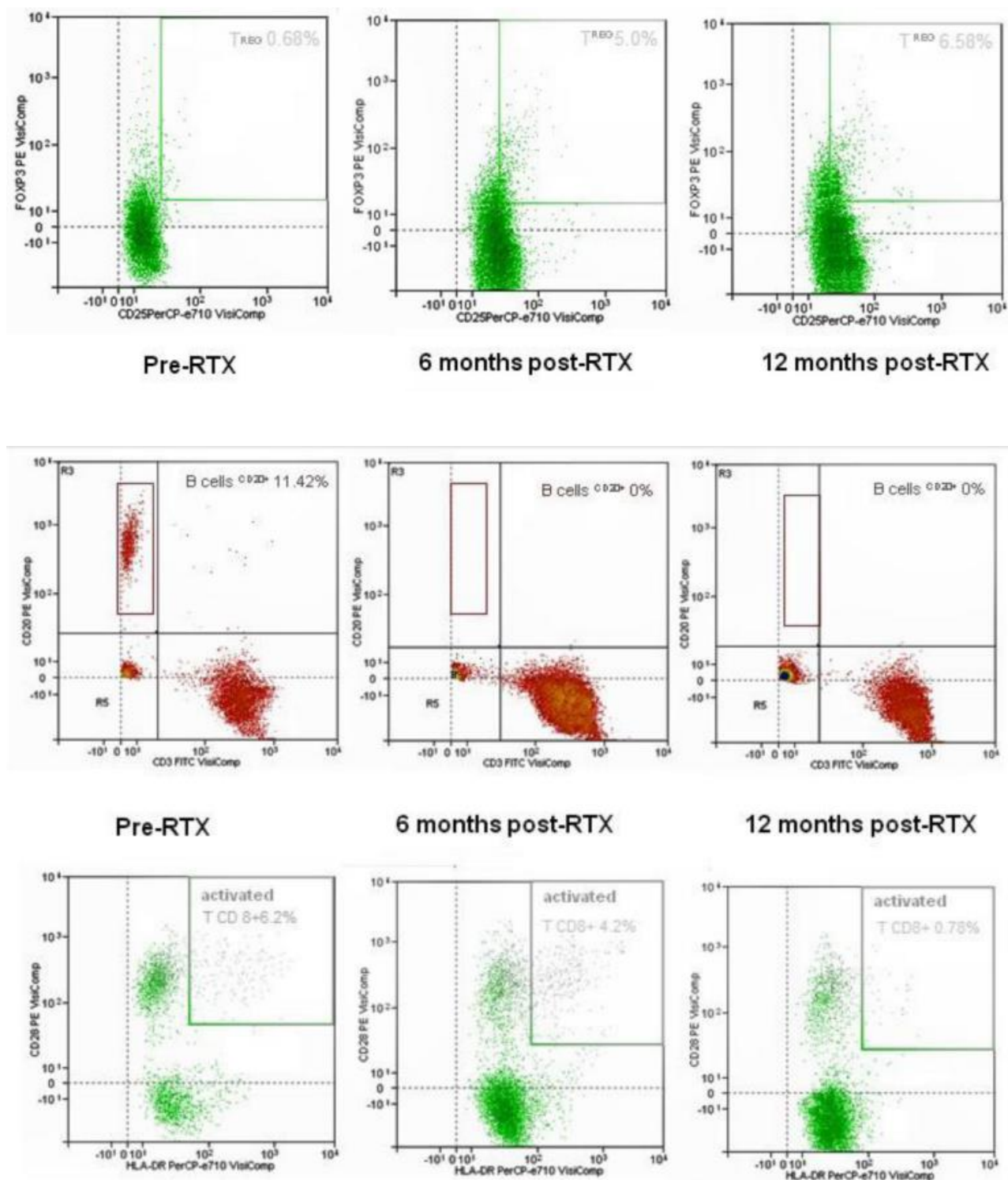


Fig. 3. Representative dot-plots of TReg (CD4+CD25+FOXP3+, top plots), and B-cell (CD 20+, central plots) and activated T CD8+ cell (CD 20+, lower plots) as evaluated by flow cytometry. Samples from a responder patient analysed before and at 6 and 12 months after Rituximab administration. Upon detection of B cell depletion, a 9-fold increase in the circulating Treg (CD4+CD25+FOXP3+) and a 7.5-

fold decrease in activated T CD8+ cells were observed over 12 months, suggesting Rituximab-induced

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