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Treatment with rituximab in patients with mixed cryoglobulinemia syndrome: Results of multicenter cohort study and review of the literature

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Abstract

Objective: Mixed cryoglobulinemia syndrome (MCs) is a systemic vasculitis characterized by multiple organ involvement due to the vascular deposition of immune-complexes, mainly the cryoglobulins. B-lymphocyte expansion represents the underlying pathological alteration frequently triggered by hepatitis C virus (HCV) infection. The treatment of MCs syndrome is generally based on antiviral drugs and/or immunosuppressors, among which rituximab, an anti-CD20 monoclonal antibody, has been usefully employed for both cutaneous and visceral MCs organ involvement. This multicenter study retrospectively evaluated the effects of rituximab in a large series of patients with active MCs. The observed results were compared to those emerging from the updated review of the literature on this topic.

Methods: The study included 87 patients (male/female 19/68, mean age 62.3 ± 11.4 SD years, mean disease duration 9 ± 6.2 SD years, HCV infection in 92% of cases) with active cryoglobulinemic vasculitis evaluated before rituximab monotherapy and after 6-month follow-up

by means of main clinico-serological parameters. A PubMed search up to May 31, 2011, was done to find published clinical studies, including case reports of MCs treated with rituximab.

Results: A significant clinical improvement was observed in a relevant percentage of cases, regardless the presence/absence of associated HCV infection; namely, complete/partial remission of pre-treatment active manifestations was observed in 74% of skin purpuric lesions, up to 87% of non-healing vasculitic leg ulcers, and 44% of the peripheral neuropathy, mainly paresthesias (patient's visual analogical scale from 62 ± 25 to 37 ± 27 ; $p < .0001$). Moreover, cryoglobulinemic nephropathy, observed in 38 patients, significantly improved in 95% of cases (serum creatinine from 1.8 ± 1.1 SD to 1.4 ± 0.8 SD mg/dl, $p < .0001$; 24-hour proteinuria from 2.2 ± 2.1 SD to 0.9 ± 1.7 SD g/24 h, $p < .0001$), with complete remission in the 50%. Among 6 patients with complicating non-Hodgkin's B-cell lymphoma a complete or partial remission was observed in 5/6. A complete remission of abdominal vasculitis was also observed in one patient. These beneficial effects were mirrored by the improvement of cryoglobulinemic serological hallmarks, namely cryocrit and low complement C4, in half cases.

The safety of rituximab was confirmed by the small number of side effects recorded during the 6-month follow-up. On the whole, the results of the present study are in keeping with those reported in 39 papers present in world literature, including a total of 279 MCs patients.

Conclusions: Rituximab may be regarded as useful and safe pathogenetic treatment of cryoglobulinemic vasculitis. The actual role of this drug should be definitely confirmed by randomized controlled trials, as well as its position in the therapeutical strategy, mainly with respect to antiviral treatment in HCV-associated MCs.

1. Introduction

Mixed cryoglobulinemia syndrome (MCs) is a systemic vasculitis characterized by multiple organ involvement due to the vascular deposition of immune-complexes, mainly mixed IgG-IgM cryoglobulins and complement [1-3]. These cryoprecipitable immune-complexes are the result of B-cell clone proliferation producing pathogenic IgM with rheumatoid factor activity. In the majority of cases the B-cell expansion is triggered by chronic hepatitis C virus (HCV) infection; the HCV lymphotropism is responsible for the immune-system alterations underlying the MCs [3,4]. Clinically, the disease may present with variable symptom composition ranging from mild MCs (purpura, arthralgia, and asthenia) to more serious manifestations; namely, neurological, renal, hepatic, and/or wide spread vascular involvement [1-5]. Patients with mild manifestations are generally responsive to symptomatic treatments; while, moderate-severe symptoms may deserve more aggressive approach with etiological and/or pathogenetic therapies, in particular antiviral or immunosuppressive drugs [5-7]. Considering the rarity of the disease in general and of clinically homogeneous patients' with active, severe cryoglobulinemic vasculitis, data regarding

etio-pathogenetic treatments are generally limited to small patients' series [6]. The combination of pegylated interferon-alpha with ribavirin may represent the first-choice treatment for more stabilized patients with ongoing HCV infection complicated by severe organ involvement, especially when associated to active chronic hepatitis [7,8].

The antiviral treatment may be limited by frequent poor tolerability and/or responsiveness, including the low rate of HCV eradication, observed in a not negligible number of patients [5,6]; in these cases, as well as in very active cases, and obviously in HCV- negative individuals the pathogenetic treatments, namely corticosteroids, cyclophosphamide, and/or plasmapheresis, represent the standard approach to cryoglobulinemic vasculitis, firstly proposed in the pre-HCV decades [5]. These therapies may be responsible for serious, seldom life-threatening complications; therefore, the management of patients with MCs is often particularly difficult [5,6]. Since 1999 anecdotal observations followed by two pilot studies showed the efficacy and safety of anti-CD20 monoclonal antibody (rituximab) treatment in patients with cryoglobulinemic vasculitis, often resistant or intolerant to other therapies [9-12]. The safety and efficacy of this drug have been reported in various vasculitides and other systemic autoimmune diseases [13-23]. The usefulness of monoclonal antibodies directed to CD20 antigen, a trans membrane protein expressed on pre-B lymphocytes and mature B lymphocytes, on both cutaneous and visceral organ involvement of MCs, including low grade B-cell lymphoma, has been reported by various Authors, generally referred to small patients' series [24-31]. These are often characterized by a heterogeneous composition of clinical manifestations that does not permit an accurate evaluation of the effect of rituximab on single organ involvement. In the present multicenter study we retrospectively analyzed a large series of patients with cryoglobulinemic vasculitis treated with rituximab; moreover, we compared the observed clinical results with those emerging from the updated review of the literature on the same topic.

2. Patients and methods

A series of 87 patients with active cryoglobulinemic vasculitis (male/female 19/68, mean age 62.3 ± 11.4 SD years, mean disease duration 9 ± 6.2 SD years) were enrolled for this multicenter retrospective study (Table 1). The patients recruited have been recently treated with rituximab but never described in previous reports. In all cases the MCs classification satisfied the recently proposed criteria [32]; concomitant autoimmune systemic diseases or malignancies were invariably ruled-out, as well as possible infectious conditions other than HCV. The possible association with hepatotropic virus infection, i.e. HCV and HBV, was investigated by detection of specific serum antibodies and viremia. Both clinical and laboratory parameters were carefully evaluated according to standard methodologies as previously described [3,5,33]. At the time of the rituximab treatment, the patients showed a variable combination of MCs clinical

manifestations (Table 1); namely, cutaneous symptoms, mainly purpura and skin ulcers, peripheral sensory-motor neuropathy, renal involvement, chronic hepatitis, widespread vasculitic syndrome, and/or B-cell lymphoma. In all cases the presence of clinically advanced cirrhosis and/or hepatocellular carcinoma was excluded. Laboratory investigations comprised serum cryoglobulin detection (cryocrit) and composition, rheumatoid factor determination, routine blood chemistry, and urinalysis [5]. When clinically required organ involvement (skin, kidney, liver, and/or bone marrow) was further investigated by means of ultrasonography and/or bioptical tissue examination. The effects of treatment were evaluated by comparing clinical and immunological parameters before rituximab cycle and at first 6 month follow-up. Clinical response to treatment was defined by evaluating the following main clinical signs: vasculitic skin involvement (variations of purpura score and/or dimensions of ulcerative lesions), peripheral sensory or sensory-motor neuropathy (clinical and/or electrophysiological changes), renal involvement (variations of serum creatinine level and/or 24-hour proteinuria), B-cell lymphoma (clinical symptoms and bone marrow infiltrate changes) [5]. Therapeutical effects of rituximab were defined as a) complete response (CR) for disappearance of baseline main manifestation(s), b) partial response (PR) for improvement of clinical manifestation (s) \geq 50% of baseline), and c) no responders (NR) for absent or insufficient response (improvement $<$ 50%) [34]. The relapse of MCs was defined as the reappearance of at least one of pre-treatment clinical signs of MCs during the first 6-month follow-up. For immunological parameters, a complete response of cryocrit was defined by the disappearance of serum cryoglobulins, while a partial response for a cryocrit decrease of more than 50% from the baseline level; in patients with abnormally reduced C4 (below lower limit of normal range), a complete response was referred to the C4 normalization and a partial response to increased C4 levels without normalization.

Severe adverse events attributable to rituximab were defined as an adverse event that occurred during infusion sessions or within the first 12 months following the treatment and that required hospitalization and/or resulted in death (or that required intravenous antibiotics in case of infection) [34]. Statistical analysis was carried out by means of non-parametrical Wilcoxon test to evaluate the variations before/after rituximab treatment.

3. Review of the literature

A PubMed search up to 31 May 2011, with the key words 'cryoglobulinemia', 'cryoglobulinemic vasculitis', 'rituximab', and 'anti-CD20', was done to find published clinical studies, including case reports of MCs treated with rituximab. Review papers were excluded. A total of 39 manuscripts that reported on at least one case of cryoglobulinemic vasculitis treated with rituximab were included in the present analysis [9-12,24-31,34-60]. Three manuscripts were excluded because they included patients that had already been described in other publications [9,11,37].

4. Results

The baseline clinico-epidemiological features of 87 patients with MCs undergoing rituximab treatment are shown in Table 1. HCV-related MCs were present in the large majority of cases (92%), while the so-called 'essential' MCs were present in only 5 (6%) patients; only 2 individuals showed MCs overlapping with other connective tissue diseases (Sjogren's syndrome in both cases). The treatment with rituximab was decided because of non-responsiveness or intolerance to other previous treatments; in only 13 (15%) patients rituximab was administered as first-line therapy for cryoglobulinemic syndrome. The main indications to rituximab were a membranoproliferative glomerulonephritis (MPGN) in 30% of cases, peripheral neuropathy in 23%, and severe skin vasculitis, including non-healing ulcers, in 25%; while 14% of individuals showed multiple active manifestations (Table 1). The administration schedule of rituximab comprised two ($1 \text{ g} \times 2$ every other week in 18 pts) or four sessions of intravenous infusions ($375 \text{ mg/m}^2/\text{week} \times 4$ in 59 pts); while 10 patients received a cycle of 6 infusions ($375 \text{ mg/m}^2/\text{week} \times 4 + 375 \text{ mg/m}^2$ monthly for 2 months). Antiviral therapy had been previously administered to 44 (50.6%) patients, and was suspended at least 6 months before rituximab administration. Concomitant treatments to rituximab were only glucocorticoids, when needed. Efficacy data were analyzed 6 months after rituximab treatment.

At the end of 6-month follow-up, the overall patients' evaluation showed a clear-cut clinical improvement in a relevant percentage of cases. In particular, CR of pre-treatment active manifestations was frequently observed, varying from the 44% of the peripheral neuropathy to 74% of purpuric lesions. Skin manifestations of cryoglobulinemic vasculitis were particularly responsive to rituximab treatment; namely, non-healing vasculitic leg ulcers gradually recovered or improved in 87% of cases. Among the most harmful complications of the disease, MPGN improved in the large majority of patients (95%), with complete remission in 50% (normalization of serum creatinine and absence of significant proteinuria). Fig. 1 shows the significant decrease of serum creatinine (from $1.8 \pm 1.1\text{SD}$ to $1.4 \pm 0.8\text{SD}$ mg/dl; $p < .0001$), 24-hour proteinuria (from $2.2 \pm 2.1\text{SD}$ to $0.9 \pm 1.7\text{SD}$ g/24 h, ($p < .0001$), as well as the improvement of paresthesias due to peripheral sensory neuropathy evaluated by patient's visual analogical scale (from 62 ± 25 to 37 ± 27 ; $p < .0001$). Among 6 patients with complicating non-Hodgkin's B-cell lymphoma a complete or partial remission was observed in 5/6. The patient with abdominal vasculitis showed a complete remission of this life-threatening clinical manifestation.

Finally a complete/partial response of cryoglobulinemic serological hallmarks, namely cryocrit and low complement C4, was observed in half cases (Table 2).

A relatively small number of side effects were recorded after rituximab treatment (Table 2).

In particular, infusion-related reactions were observed in 4 patients, i.e. hypotension (2), urticaria (1), and serum sickness-like reaction (1). During the 6-month follow-up four patients developed infectious complications, including urinary tract infection (2), infectious pneumonia (1), and gangrene (1) or mild/transient symptoms such as neutropenia (2), hypogammaglobulinemia (5), hypertransaminasemia (1). Three patients experienced severe adverse events, namely infectious pneumonia, gangrene, and the above mentioned serum sickness-like reaction. For these reasons, these three patients dropped-out; another patient discontinued the treatment due to worsening of vasculitis.

On the whole, rituximab might be considered responsible for disease worsening in only 2/87 (2.2%) patients; one developed a severe peripheral neuropathy, the other one showed a worsening of necrotizing skin vasculitis.

5. Review of the literature

Including the first single case report described by Zaja et al. in 1999 [9-12,24-31,34-60], a total of 40 manuscripts that reported on at least one case of cryoglobulinemic vasculitis treated with rituximab were considered in the present analysis (Table 3). Besides the present case series, Table 3 includes a total of 279 patients with MCs described between 1999 and 2011; it regards numerous case reports and 16 clinical studies with at least 5 patients treated with rituximab (Table 3). No controlled clinical trials have been published to date, with the exception of one recent study [61]. There is a great inhomogeneity among different series of patients treated with rituximab as regards to both previous therapeutical approaches and indications to treatment. In this respect, the most frequent manifestations needing rituximab therapy were skin vasculitis, peripheral neuropathy, and renal involvement. The majority of patients (84.8%) showed HCV-associated cryoglobulinemic vasculitis, often characterized by type II mixed cryoglobulinemia (88%). The cumulative dosage of rituximab cycles and treatment modalities were quite uniform; generally the schedule included four consecutive intravenous infusions of 375 mg/m²/week of rituximab (69%). In 3 recent studies [26,29,30], the rituximab was associated to antiviral therapy (Peg-interferon-alpha + ribavirin), while in one with other immunosuppressive or antineoplastic drugs [34] (Table 3). After the first 6-month follow-up, a clear-cut clinical improvement of main cryoglobulinemic manifestations was observed in the majority of cases (Table 3). With the limitations due to the above mentioned inhomogeneity among treated patients' series, the cumulative analysis of previous studies reporting detailed therapeutical effects showed a CR or PR for skin vasculitis in 106/117 (94%), skin ulcers in 32/36 (89%), peripheral neuropathy in 85/89 (95%), renal involvement in 46/52 (88%), B-NHL in all reported cases, and remission of abdominal vasculitis in 7/8 (87%) patients. Severe adverse effects possibly related to anti-CD20 treatment were recorded in 18/252 (7%) subjects (Table 3).

6. Discussion

This multicenter, retrospective study including a large patients' series further supports and expands the results of previous reports showing the usefulness of rituximab in active MCs [9-12,24-31,34-60]. The positive effects of this treatment were particularly evident for the most frequent disease manifestations such cutaneous vasculitic lesions, i.e. severe purpura and/or non-healing ulcers, and peripheral neuropathy, often resistant to traditional treatments. Moreover, the number of patients recruited permitted to better evaluate the effects of anti-CD20 therapy on other less frequent complications, such as renal involvement that represents a harmful visceral complication of cryoglobulinemic vasculitis [1-3,5,33]. The large majority of individuals (95%) showed a complete or partial remission of MPGN at 6-month follow-up from rituximab cycle. On the whole, the significant clinical improvement was mirrored by the positive variations of serological MCs hallmarks in half cases; namely, the reduction of circulating cryoglobulin levels and/or the increase/normalization of complement C4. Together with the high percentage of patients responsive to rituximab, the low rate of side effects confirmed the safety of this drug, during the treatment sessions as well as the entire 6-month follow-up. This was also the case of liver damage; the absence of any signs of hepatic toxicity is remarkable considering the association of MCs with chronic HCV infection in the large majority of patients (92%). In this respect, previous studies clearly demonstrated that, when present, the increase of HCV viral load did not affect the liver tests as well as other clinico-serological parameters [11,12]. The lack of hepatic toxicity has been further evidenced by a recent clinical observation in patients with HCV-related MCs and advanced cirrhosis treated by rituximab [29,49]; besides its efficacy on cryoglobulinemic vasculitis the treatment was followed by an improvement of liver function. It is possible to ascribe this unexpected hepatic amelioration to the depletion of CD20+ B-cells induced by rituximab through two different mechanisms; firstly, the drug may reduce also the hepatic B-cell infiltrates conditioning liver damage and, indirectly, it may improve the Kupffer cell function as consequence of serum cryoglobulin level reduction [28]. Apart from the efficacy and the long-term safety of rituximab, Sène and Colleagues reported a significant number of systemic reactions after the drug infusion [62]. In HCV-related cryoglobulinemic vasculitis, rituximab may form a complex with RF-positive IgM, responsible for accelerated cryoprecipitation and severe systemic reactions. To avoid such harmful infusion-related complication, rituximab should be administered with caution in cryoglobulinemic vasculitis [62]. Even if serum sickness has been rarely observed in the large majority of studies with rituximab in MCs [6], French authors suggested the use of protocol with four weekly consecutive intravenous infusions of 375 mg/m² of rituximab, possibly associated with plasmapheresis prior to rituximab infusion, mainly in patients with high baseline levels of mixed cryoglobulins [62]. However, we observed a low rate of infusion-related complications, both in patients treated with this regimen, and in patients treated with different rituximab schedule of 1 gram every two weeks (two infusions in total).

The natural history of MCs is unpredictable and strongly depends on the severity of disease complications and co-morbidities, as well as the response to available treatments. Morbidity due specifically to cryoglobulinemic syndrome may also be significant; it may be related to infections, cardiovascular diseases, progressive renal failure, and advanced neuropathy [5]. The overall prognosis is worse in patients with renal disease, liver failure, lymphoproliferative disease, and malignancies [5]. Therefore, a careful monitoring of life-threatening symptoms of MCs, mainly nephropathy and widespread vasculitis, as well as endocrine, metabolic, vasculopathic, and neoplastic complications, such as B-cell lymphoma, should be carried out in all patients [33].

The treatment of MCs is particularly challenging; it is mainly due to the complex etiopathogenesis of the disease characterized by the coexistence of chronic HCV infection, autoimmune/lymphoproliferative alterations, and immune-complex-related vasculitis [5,33,63]. Because of the clinical polymorphism of the disease, clinicians of different medicine subspecialties, such as rheumatologists, hepatologists, nephrologists, etc., are generally involved in the management of various clinical phenotypes of cryoglobulinemic syndrome. Consequently, there is not a unanimously accepted therapeutic strategy among various authors [5,6]. In this context, the role of rituximab in the therapeutical armamentarium of cryoglobulinemic vasculitis is progressively increased in the last decade. The main target of this drug is the B-lymphocyte proliferation that represents a crucial step in the complex etiopathogenesis of the disease [9-12,24-31,34-60]. In HCV-positive individuals, the therapeutical intervention should be directed at chronic HCV infection, which may trigger and possibly maintain the disease, the T- and B-lymphocyte auto-reactivity with prominent clonal B-cell expansion, and finally at the small vessel vasculitis due to circulating cryo- and non-cryoprecipitable immune-complexes [5,6,33]. According to this pathogenetic cascade, the disease can be treated at different levels by means of etiologic, pathogenetic, and/or symptomatic therapies [33]. In HCV-related MCs an attempt at HCV eradication by alpha-interferon and ribavirin treatment should be done, mainly in patients with clinically relevant liver involvement and/or recent onset of cryoglobulinemic vasculitis [33]. The antiviral treatment shows frequent dropouts, including important immune-mediated side effects such as peripheral sensory-motor neuropathy, thyroiditis, and rheumatoid-like polyarthritis [33]. The lack of HCV eradication, mainly in patients with HCV-genotype 1, or in the presence of stabilized self-perpetual autoimmune disorder the beneficial effect observed with the antiviral treatment may be weak or transient. With regards the first-line treatment of HCV-related MCs, the expert opinions are quite divergent; given the lack of randomized controlled trials for both antiviral and immunosuppressive drugs, including rituximab, it is very difficult to define a standard therapeutical strategy [6]. The tailoring of treatment by focusing on the single patient's clinical history, disease manifestations, and possible co-morbidities is generally the preferred approach. In cryoglobulinemic patients with life-threatening manifestations, including B-cell neoplasias, and in those with 'essential' MCs the immunomodulating/immunosuppressive treatments represent the first-line intervention. As for the classical systemic vasculitides, the presence of severe, rapidly progressive vasculitic complications needs combined treatment with high-dose steroids, immunosuppressors, and plasma exchange [6,29]. In the setting of severe/active, but not life-threatening conditions rituximab might now represent the first option in

most instances [9-12,24-31,34-60]; conversely, antiviral treatment with pegylated interferon-alpha and ribavirin should be employed for more stabilized patients with ongoing HCV infection and severe organ involvement, particularly active chronic hepatitis [6,33].

Some recent clinical studies suggested that sequential or combined antiviral and immunosuppressive (rituximab) treatment could represent a rather useful therapeutic strategy in patients with active MCs [26,29,30]. The rationale of such aggressive therapy could be particularly indicated in HCV-related MCs with major clinical manifestations and/or poor response to standard treatments. In many patients with mild to moderate symptoms the use of long-term steroid treatment is often responsible for important side effects and co-morbidities, mainly in older patients with long-lasting MCs. In these conditions, intermittent rituximab cycles may be usefully employed by reducing/avoiding the chronic steroid administration [43].

7. Conclusions

On the whole, we can affirm that rituximab may be regarded as useful and safe treatment of cryoglobulinemic vasculitis; regardless the presence/absence of associated HCV infection. The actual role of this drug and its position in the therapeutical strategy, should be confirmed by randomized controlled trials.

Take-home messages

- The treatment of MCs is based on etiological, pathogenetic, and/or symptomatic therapies.
- Among pathogenetic treatments, rituximab is increasingly used during the last decade.
- Our study shows the efficacy and safety of rituximab in a series 87 MCs patients.
- Skin lesions, neuropathy, and nephropathy were particularly responsive to rituximab.
- An updated review of published reports on this topic is done.

References

- [1] Gorevic PD, Kassab HJ, Levo Y, Kohn R, Meltzer M, Prose P, et al. Mixed cryoglobulinemia: clinical aspects and long-term follow-up of 40 patients. *Am J Med* 1980;69:287-308.
- [2] Brouet JC, Clauvel JP, Danon F, Klein M, Seligmann M. Biologic and clinical significance of cryoglobulins. A report of 86 cases. *Am J Med* 1974;57:775-88.
- [3] Ferri C, Zignego AL, Pileri SA. Cryoglobulins. *J Clin Pathol* 2002;55:4-13.
- [4] Ferri C, Monti M, La Civita L, Longombardo G, Greco F, Pasero G, et al. Infection of peripheral blood mononuclear cells by hepatitis C virus in mixed cryoglobulinemia. *Blood* 1993;82:3701-4.
- [5] 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.
- [6] Pietrogrande M, De Vita S, Zignego AL, Pioltelli P, Sansonno D, Sollima S, et al. Recommendations for the management of mixed cryoglobulinemia syndrome in hepatitis C virus-infected patients. *Autoimmun Rev* 2011;10:444-54.
- [7] Saadoun D, Resche-Rigon M, Thibault V, Piette JC, Cacoub P. Antiviral therapy for hepatitis C virus-associated mixed cryoglobulinemia vasculitis: a long-term followup study. *Arthritis Rheum* 2006;54:3696-706.
- [8] Mazzaro C, Pozzato G, Zorat F, Baragiotta AM, Biscontin MG, Spina M, et al. Etiologic treatment of hepatitis C virus-associated mixed cryoglobulinemia. *Dig Liver Dis* 2007;39:S102-6.
- [9] Zaja F, Russo D, Fuga G, Patriarca F, Ermacora A, Baccarani M. Rituximab for the treatment of type II mixed cryoglobulinemia. *Haematologica* 1999;84:1157-8.
- [10] Arzoo K, Sadeghi S, Liebman HA. Treatment of refractory antibody mediated autoimmune disorders with an anti-CD20 monoclonal antibody (rituximab). *Ann Rheum Dis* 2002;61:922-4.
- [11] Zaja F, De Vita S, Russo D, Michelutti A, Fanin R, Ferraccioli G, et al. Rituximab for the treatment of type II mixed cryoglobulinemia. *Arthritis Rheum* 2002;46: 2252-4.
- [12] Sansonno D, De Re V, Lauletta G, Tucci FA, Boiocchi M, Dammacco F. Monoclonal antibody treatment of mixed cryoglobulinemia resistant to interferon alpha with an anti-CD20. *Blood* 2003;101:3818-26.
- [13] Engel P, Gómez-Puerta JA, Ramos-Casals M, Lozano F, Bosch X. Therapeutic targeting of B cells for rheumatic autoimmune diseases. *Pharmacol Rev* 2011;63: 127-56.
- [14] Wang C, Pan HF, Ye DQ. The therapeutic potential of the targeted autoreactive B lymphocytes by rituximab in SLE. *Autoimmun Rev* 2011 [Epub ahead of print].

- [15] Bosello S, De Luca G, Tolusso B, Lama G, Angelucci C, Sica G, et al. B cells in systemic sclerosis: a possible target for therapy. *Autoimmun Rev* 2011 [Epub ahead of print].
- [16] Perosa F, Prete M, Racanelli V, Dammacco F. CD20-depleting therapy in autoimmune diseases: from basic research to the clinic. *J Intern Med* 2010;167: 260-77.
- [17] Scoble T, Wijetilleka S, Khamashta MA. Management of refractory anti-phospholipid syndrome. *Autoimmun Rev* 2011 [Epub ahead of print].
- [18] Marie I, Mouthon L. Therapy of polymyositis and dermatomyositis. *Autoimmun Rev* 2011 [Epub ahead of print].
- [19] Kieseier BC, Lehmann HC, Horste GM. Autoimmune diseases of the peripheral nervous system. *Autoimmun Rev* 2011 [Epub ahead of print].
- [20] Rutgers A, Kallenberg CG. Refractory vasculitis. *Autoimmun Rev* 2011 [Epub ahead of print].
- [21] Fabris M, Quartuccio L, Lombardi S, Saracco M, Atzeni F, Carletto A, et al. The CC homozygosity of the $\square 174$ G N C IL-6 polymorphism predicts a lower efficacy of rituximab therapy in rheumatoid arthritis. *Autoimmun Rev* 2010 [Epub ahead of print].
- [22] Galarza-Maldonado C, Kourilovitch MR, Molineros JE, Cardiel MH, Zurita L, Soroka NF, et al. The administration of low doses of rituximab followed by hydroxy-chloroquine, prednisone and low doses of mycophenolate mofetil is an effective therapy in Latin American patients with active systemic lupus erythematosus. *Autoimmun Rev* 2010;10:108-11.
- [23] Conti F, Perricone C, Ceccarelli F, Valesini G. Rituximab treatment of systemic lupus erythematosus in controlled trials and in clinical practice: two sides of the same coin. *Autoimmun Rev* 2010;9:716-20.
- [24] De Vita S, Quartuccio L, Fabris M. Rituximab in mixed cryoglobulinemia: increased experience and perspectives. *Dig Liver Dis* 2007;39:S122-8.
- [25] Roccatello D, Baldovino S, Rossi D, Giachino O, Mansouri M, Naretto C, et al. Rituximab as a therapeutic tool in severe mixed cryoglobulinemia. *Clin Rev Allergy Immunol* 2008;34:111-7.
- [26] Saadoun D, Resche-Rigon M, Sene D, Perard L, Karras A, Cacoub P. Rituximab combined with Peg-interferon-ribavirin in refractory hepatitis C virus-associated cryoglobulinaemia vasculitis. *Ann Rheum Dis* 2008;67:1431-6.
- [27] Cavallo R, Roccatello D, Menegatti E, Naretto C, Napoli F, Baldovino S. Rituximab in cryoglobulinemic peripheral neuropathy. *J Neurol* 2009;256:1076-82.

- [28] Petrarca A, Rigacci L, Caini P, Colagrande S, Romagnoli P, Vizzutti F, et al. Safety and efficacy of rituximab in patients with hepatitis C virus related mixed cryoglobulinemia and severe liver disease. *Blood* 2010;116:335-42.
- [29] Saadoun D, Resche Rigon M, Sene D, Terrier B, Karras A, Perard L, et al. Rituximab plus Peg-interferon-alpha/ribavirin compared with Peg-interferon-alpha/ribavirin in hepatitis C-related mixed cryoglobulinemia. *Blood* 2010;116:326-34.
- [30] Dammacco F, Tucci FA, Lauletta G, Gatti P, De Re V, Conteduca V, et al. Pegylated interferon-alpha, ribavirin, and rituximab combined therapy of hepatitis C virus related mixed cryoglobulinemia: a long-term study. *Blood* 2010;116:343-53.
- [31] Visentini M, Ludovisi S, Petrarca A, Pulvirenti F, Zaramella M, Monti M, et al. A phase II, single-arm multicenter study of low-dose rituximab for refractory mixed cryoglobulinemia secondary to hepatitis C virus infection. *Autoimmun Rev* 2011, doi:10.1016/j.autrev.2011.04.033.
- [32] De Vita S, Soldano F, Isola M, Monti G, Gabrielli A, Tzioufas A, et al. Preliminary classification criteria for the cryoglobulinaemic vasculitis. *Ann Rheum Dis* 2011;70:1183-90.
- [33] Ferri C. Mixed cryoglobulinemia. *Orphanet J Rare Dis* 2008;3:25.
- [34] Terrier B, Launay D, Kaplanski G, Hot A, Larroche C, Cathébras P, et al. Safety and efficacy of rituximab in non-viral cryoglobulinemia vasculitis: data from the French AIR registry. *Arthritis Care Res* 2010;62:1787-95.
- [35] Zaja F, De Vita S, Mazzaro C, Sacco S, Damiani D, De Marchi G, et al. Efficacy and safety of rituximab in type II mixed cryoglobulinemia. *Blood* 2003;101:3827-34.
- [36] Lamprecht P, Lerin-Lozano C, Merz H, Dennin RH, Gause A, Voswinkel J, et al. Rituximab induces remission in refractory HCV associated cryoglobulinaemic vasculitis. *Ann Rheum Dis* 2003;62:1230-3.
- [37] Roccatello D, Baldovino S, Rossi D, Mansouri M, Naretto C, Gennaro M, et al. Long term effects of anti-CD20 monoclonal antibody treatment of cryoglobulinaemic glomerulonephritis. *Nephrol Dial Transplant* 2004;19:3054-61.
- [38] Ghobrial IM, Uslan DZ, Call TG, Witzig TE, Gertz MA. Initial increase in the cryoglobulin level after rituximab therapy for type II cryoglobulinemia secondary to Waldenstrom macroglobulinemia does not indicate failure of response. *Am J Hematol* 2004;77:329-30.
- [39] Ghijssels E, Lerut E, Vanrenterghem Y, Kuypers D. Anti-CD20 monoclonal antibody (rituximab) treatment for hepatitis C-negative therapy-resistant essential mixed cryoglobulinemia with renal and cardiac failure. *Am J Kidney Dis* 2004;43:e34-8.

- [40] Catuogno M, Rezai S, Priori R, Magrini L, Valesini G. Serum sickness associated with rituximab in a patient with hepatitis C virus-related mixed cryoglobulinaemia. *Rheumatology (Oxford)* 2005;44:406.
- [41] Basse G, Ribes D, Kamar N, Mehrenberger M, Esposito L, Guitard J, et al. Rituximab therapy for de novo mixed cryoglobulinemia in renal transplant patients. *Transplantation* 2005;80:1560-4.
- [42] Koukoulaki M, Abeygunasekara SC, Smith KG, Jayne DR. Remission of refractory hepatitis C-negative cryoglobulinaemic vasculitis after rituximab and infliximab. *Nephrol Dial Transplant* 2005;20:213-6.
- [43] Quartuccio L, Soardo G, Romano G, Zaja F, Scott CA, De Marchi G, et al. Rituximab treatment for glomerulonephritis in HCV-associated mixed cryoglobulinaemia: efficacy and safety in the absence of steroids. *Rheumatology (Oxford)* 2006;45:842-6.
- [44] Cai FZ, Ahern M, Smith M. Treatment of cryoglobulinemia associated peripheral neuropathy with rituximab. *J Rheumatol* 2006;33:1197-8.
- [45] Cohen H, Green S, Jones S, Amos N, William BD. Lack of efficacy of Rituximab in a patient with essential mixed cryoglobulinaemia. *Rheumatology* 2007;46:366-7.
- [46] Braun A, Neumann T, Oelzner P, Hein G, Gröne HJ, Ziemer M, et al. Cryoglobulinaemia type III with severe neuropathy and immune complex glomerulonephritis: remission after plasmapheresis and rituximab. *Rheumatol Int* 2008;28: 503-6.
- [47] Rego TC, Massumoto CM, Batista RS, Moura LH, Soares LM, Gomes AP. The use of monoclonal antibody (rituximab) in the treatment of type II mixed cryoglobulinemia. *Braz J Infect Dis* 2007;11:174-5.
- [48] Visentini M, Granata M, Veneziano ML, Borghese F, Carlesimo M, Pimpinelli F, et al. Efficacy of low-dose rituximab for mixed cryoglobulinemia. *Clin Immunol* 2007;125:30-3.
- [49] Petrarca A, Rigacci L, Monti M, Giannini C, Bernardi F, Caini P, et al. Improvement in liver cirrhosis after treatment of HCV-related mixed cryoglobulinemia with rituximab. *Dig Liver Dis* 2007;39:S129-33.
- [50] Massari M, Catania A, Magnani G. Efficacy and risk of rituximab in type II mixed cryoglobulinemia: a significant case report. *Dig Liver Dis* 2007;39:S134-5.
- [51] Korte MR, van Heerde MJ, de Man RA, Betjes MH. Rituximab for the treatment of glomerulonephritis in hepatitis C associated cryoglobulinaemia. *Neth J Med* 2008;66:27-30.
- [52] da Silva Fucuta Pereira P, Lemos LB, de Oliveira Uehara SN, de Souza E, Silva IS, Silva AE, Ferraz ML. Long-term efficacy of rituximab in hepatitis C virus-associated cryoglobulinemia. *Rheumatol Int* 2009;30:1515-8.

- [53] Pasa S, Altintas A, Cil T, Danis R, Ayyildiz O, Muftuoglu E. A case of essential mixed cryoglobulinemia and associated acquired von-Willebrand disease treated with rituximab. *J Thromb Thrombolysis* 2009;27:220-2.
- [54] Tallarita T, Gagliano M, Corona D, Giuffrida G, Giaquinta A, Zerbo D, et al. Successful combination of Rituximab and plasma exchange in the treatment of cryoglobulinemic vasculitis with skin ulcers: a case report. *Cases J* 2009;2:7859.
- [55] Ruch J, McMahon B, Ramsey G, Kwaan HC. Catastrophic multiple organ ischemia due to an anti-Pr cold agglutinin developing in a patient with mixed cryoglobulinemia after treatment with rituximab. *Am J Hematol* 2009;84:120-2.
- [56] Quartuccio L, Petrarca A, Mansutti E, Pieroni S, Calcabrini L, Avellini C, et al. Efficacy of rituximab in severe and mild abdominal vasculitis in the course of mixed cryoglobulinemia. *Clin Exp Rheumatol* 2010;28:s84-7.
- [57] Baerlecken NT, Schmidt RE. Essential mixed cryoglobulinemia type III with leukocytoclastic vasculitis: remission by rituximab. *Clin Rheumatol* 2010, doi: 10.1007/s10067-010-1412-8.
- [58] Cabibbo S, Antolino A, Garozzo G, Manenti GO, Bonomo P. Clinical improvement induced by rituximab in two cases of type II mixed cryoglobulinaemia syndrome unresponsive to conventional treatments. *Blood Transfus* 2010;8:196-8.
- [59] Uppal R, Charles E, Lake-Bakaar G. Acute wrist and foot drop associated with hepatitis C virus related mixed cryoglobulinemia: rapid response to treatment with rituximab. *J Clin Virol* 2010;47:69-71.
- [60] Wink F, Houtman PM, Jansen TL. Rituximab in cryoglobulinaemic vasculitis, evidence for its effectivity: a case report and review of literature. *Clin Rheumatol* 2011;30:293-300.
- [61] De Vita S, Quartuccio L, Isola M, Masolini P, Sacco S, De Marchi G, et al. A randomized, controlled, multicenter phase III study of the efficacy and safety of rituximab (RTX) monotherapy versus the best available treatment in patients with mixed cryoglobulinemia syndrome. *Arthritis Rheum* 2011;62:S924.
- [62] Sène D, Ghillani-Dalbin P, Amoura Z, Musset L, Cacoub P. Rituximab may form a complex with IgMkappa mixed cryoglobulin and induce severe systemic reactions in patients with hepatitis C virus-induced vasculitis. *Arthritis Rheum* 2009;60: 3848-55.
- [63] Zignego AL, Ferri C, Pileri SA, Caini P, Bianchi FB. Extrahepatic manifestations of Hepatitis C Virus infection: a general overview and guidelines for a clinical approach. *Dig Liver Dis* 2007;39:2-17.

Anti-muscarinic acetylcholine receptor peptides antibodies in Sjögren's Syndrome: small bricks in the mystery

The diagnosis of Sjögren's Syndrome (SS) is still puzzling, and the clinical significance of pathogenic autoantibodies remains controversial. Nonetheless, anti-muscarinic acetylcholine type-3 receptor (anti-M3R) autoantibodies have been shown to be a good serum marker in the disease, since the first report from Gao et al (Arthritis Rheum. 2004;50:2615-21). The exact epitope against which the autoimmune response is directed is still not clear, thus, he et al assessed the clinical correlations of anti-M3R-derived peptide antibodies in patients with SS (Rheumatology (Oxford). 2011;50:879-84). In an elegant paper, the authors synthesized four cyclic peptides (namely, cyclic1M3R peptide (c1M3RP), c2M3RP, c3M3RP, c4M3RP) and then performed a solid-phase immunoassay for M3RP. The results showed that the cyclic 2 M3R peptide had a prevalence of 62.2% in patients with SS, significantly higher than that observed in patients with rheumatoid arthritis, systemic lupus erythematosus and healthy controls. Furthermore, such prevalence was higher in SS patients lacking anti-SSA and anti-SSB antibodies. It could be suggested to use this serological marker in addition with anti-SSA and anti-SSB in the diagnosis of SS. Furthermore, the anti- c2M3RP antibodies were found to be associated with higher ESSDAI score. All these data are a confirmation of an involvement of these autoantibodies in the pathogenesis of SS. The test used by He et al. for the detection of anti-c2M3RP should be replicated in wider studies, and eventually adopted in the diagnosis and monitoring of SS. However, the mechanisms by which these autoantibodies play their role in SS should be one of the challenges of the next future research.