Flare of a mixed cryoglobulinaemic vasculitis after obinutuzumab infusion

G. Martin de Fremont[1,2], A. Chiron[3,4], R. Krzysiek[2,3,6], S. Hacein-Bey-Abina[3,4], X. Mariette[1,2,5], G. Nocturne[1,2,5]

[1]*Rheumatology, AP-HP, Bicêtre Hospital, Le Kremlin Bicêtre;*
[2]*Université Paris-Saclay;*
[3]*Clinical Immunology Laboratory, AP-HP, Bicêtre Hospital, Le Kremlin Bicêtre;*
[4]*UTCBS, CNRS UMR 8258, INSERM U1267, Faculté de Pharmacie de Paris, Université de Paris;*
[5]*Centre for Immunology of Viral Infections and Autoimmune Diseases (IMVA), Institut pour la Santé et la Recherche Médicale (INSERM) UMR 1184, Université Paris-Saclay, Le Kremlin-Bicêtre;*
[6]*INSERM UMR-996, Clamart, France.*

Grégoire Martin de Fremont, MD
Andrada Chiron, MD
Roman Krzysiek, MD, PhD
Salima Hacein-Bey-Abina, MD, PhD
Xavier Mariette, MD, PhD
Gaetane Nocturne, MD, PhD

Please address correspondence to:
Gaetane Nocturne,
Rheumatology, Hôpital Bicêtre,
78 rue du Général Leclerc,
94275 Le Kremlin Bicêtre, France.
E-mail: gaetane.nocturne@aphp.fr

Received on September 18, 2020; accepted in revised form on October 19, 2020.

Clin Exp Rheumatol 2021; 39 (Suppl. 129): S52-S55.

Key words: obinutuzumab, rituximab, cryoglobulinaemic vasculitis, Sjögren's syndrome

Competing interests: none declared.

ABSTRACT

Objective. *Obinutuzumab (OBZ) is a new humanised type II anti-CD20 monoclonal antibody (mAb) approved in onco-haematology. Its use as an alternative to rituximab (RTX) in case of immunisation in autoimmune diseases has not been fully assessed yet. Here we report the case of a patient suffering from a refractory cryoglobulinaemic vasculitis (CV) associated to Sjögren's syndrome (SS) and treated with OBZ.*

Methods. *Since the patient was immunised against RTX, she was treated with OBZ at relapse. Three days after the infusion of OBZ, she presented a vasculitis flare. Rheumatoid factor level, complement level and cryoprecipitation were evaluated on consecutive serum samples of the patients and after RTX and OBZ addition in vitro.*

Results. *No evidence for cross-reactivity between anti-RTX Abs and OBZ was found. However, we could observe* in vitro *that cryoprecipitation was worsened by the simultaneous presence of anti-RTX Abs and RTX. We suggest that the flare of CV after OBZ infusion could be linked to a large release of immune complexes following B cells lysis induced by OBZ.*

Conclusion. *Based on our report, we think that the use of OBZ needs to be carefully discussed in patients with mixed CV.*

Introduction

Immunisation against rituximab (RTX) is more frequent in systemic autoimmune diseases than in rheumatoid arthritis and may cause infusion reactions or loss of efficacy. Immunised patients might be treated efficiently and safely with ofatumumab, a human type I anti-CD20 monoclonal antibody (Ab) (1). Exceptional cross-reactivity has been described between anti-RTX Abs and ofatumumab (2). However, ofatumumab is no longer available. A second alternative to RTX is obinutuzumab (OBZ), a humanised type II anti-CD20 mAb, responsible *in vitro* for higher cell death induction, antibody-dependent cell-mediated cytotoxicity and phagocytosis but less complement-dependent cytotoxicity compared to type I anti-CD20 Ab (3). In chronic lymphocytic leukaemia and non-Hodgkin's lymphoma, although more effective in inducing direct B cells depletion *in vivo*, OBZ shows more side effects (4). OBZ has already been successfully used in anti-phospholipase A2 receptor-associated membranous nephropathy (5) and is under evaluation in lupus nephritis (NCT02550652) but data are still scarce in autoimmune diseases and missing in cryoglobulinaemic vasculitis (CV) (6). Here we report the case of a patient suffering from a refractory type II mixed CV associated to Sjögren's syndrome (SS) immunised against RTX presenting a flare of the vasculitis following OBZ infusion.

Materials and methods

Case. A 66-year-old woman with a history of refractory type II mixed CV (IgM kappa) associated to SS was admitted for a relapse of purpura. Her previous lines of treatment included cyclophosphamide, azathioprine, RTX (with secondary failure due to immunisation), mycophenolate mofetil and high dose glucocorticoids (GC) (Fig. 1). Due to immunisation against RTX, she had received ofatumumab (3 infusions, respectively 3, 2 and 1.5 years prior to her admission) with good efficacy and tolerance. Cryoglobulinaemia and rheumatoid factor (RF) were positive throughout the course of the disease. At relapse, the patient showed necrotising purpura, no renal involvement, positive cryoglobulinaemia, high level of RF (1450 IU/mL), low com-

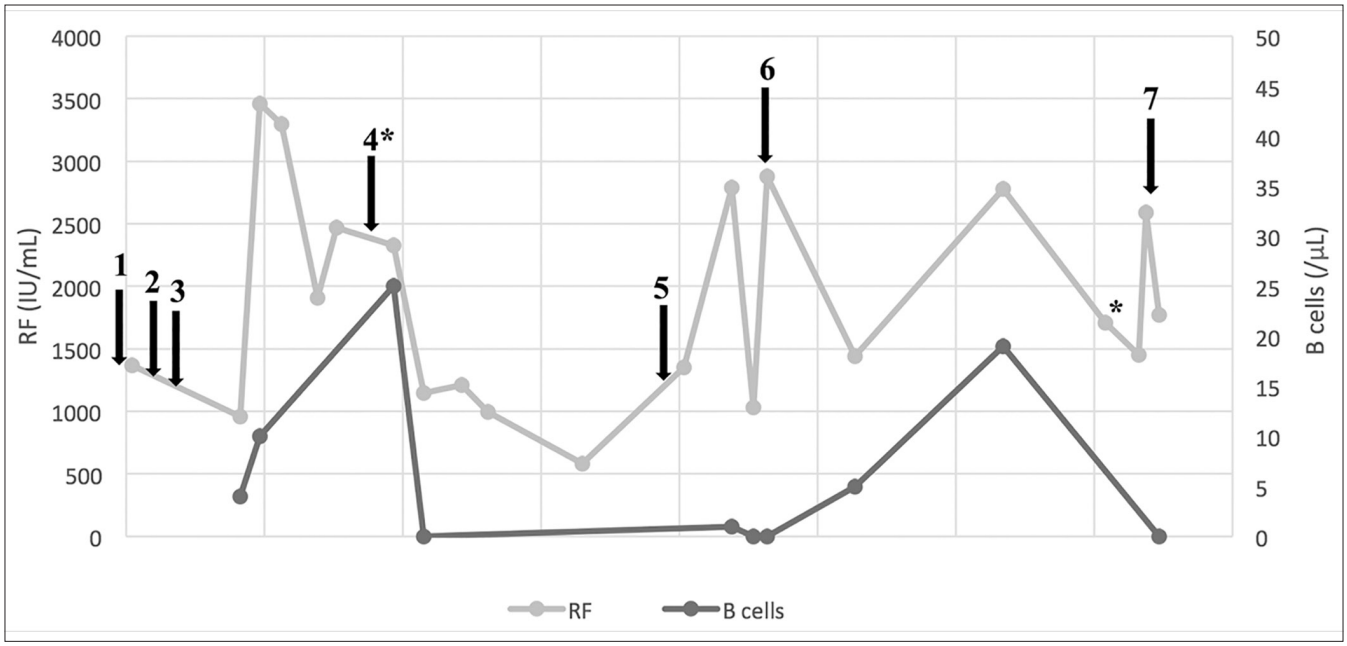

Fig. 1. Therapeutic strategies and evolution of RF and B cells.
1. Rituximab, 2. Cyclophosphamide and GC, 3. Cyclophosphamide and GC followed by mycophenolate mofetil maintenance treatment, 4. Cyclophosphamide, ofatumumab and GC followed by azathioprine and GC maintenance treatment, 5. Ofatumumab, 6. Ofatumumab,7. Obinutuzumab.
*positive anti-RTX Abs dosing.

plement C4 level (0.1 g/L) and detectable anti-RTX Abs (7 ng/mL, positivity threshold of 5 ng/mL), last B-cell count was 19 cells/μL. She received one infusion of OBZ 1g with no immediate hypersensitivity reaction. At day 3, she presented with a dramatic extension of her purpuric and necrotising lesions. Post-flare blood tests showed stable creatinine, complete peripheral B cell depletion, persistence of positive cryoglobulinaemia, stable RF and C4 levels (1040 IU/mL and <0.06 g/L, respectively) and undetectable anti-RTX Abs. The patient improved upon GC pulse but developed GC dependence and was eventually switched to belimumab (anti-BAFF mAb), which led to remission of the vasculitis symptoms.

Laboratory parameters

Complement and rheumatoid factor (RF) concentrations were assessed on the BN Prospec nephelometer (Siemens Dade-Behring, Marburg, Germany). Serum cryoglobulin detection was realised by visual inspection of cryoprecipitate formation during one month of cold incubation (4°C) and immunotyping of washed precipitate was performed using agarose gel electrophoresis followed by immunofixation (agarose 4IF Hydragel, Hydrasis 2, Sebia, Evry, France). RF was used as a surrogate to quantify cryoprecipitation since type II mixed CV is a cryoprecipitable RF. Absolute B cell counts were assessed with BD Trucount™ Tubes, on the FACS Canto II (Becton Dickinson) flow cytometer. Anti-RTX Abs were measured using Lisa-Tracker bridging-type ELISA assay (Theradiag, Marne la Vallée, France).

In vitro cryoprecipitation tests

Routine-care collected serum samples were used. Sera collected prior to RTX treatment were stored at -30°C. *In vitro* cryoprecipitate formation at 4°C and its physical characteristics were assessed at day 7 after addition of RTX or OBZ both at a final concentration of 0.5 mg/mL and compared to a negative control (*i.e.* without mAb addition) and to a serum from a patient with mixed cryoglobulinaemia but without anti-RTX Abs. C3, C4 and RF levels were measured in supernatants from all tubes and compared to baseline measures (*i.e.* before RTX/OBZ addition).

Statistical analysis

RF levels were analysed with GraphPad Prism 7.0, using Kruskal Wallis test. C3 complement levels were analysed with GraphPad Prism 7.0, using one-way ANOVA followed by Dunnet's test (Fig. 2).

Results

To better understand the reaction presented by the patient, we performed *in vitro* experiments. First, we have checked for a potential cross reactivity between pre-existing anti-RTX Abs and OBZ. We could not completely eliminate this hypothesis since there is no available assay for anti-OBZ Ab detection. However, the complete peripheral B cell depletion observed 3 weeks after the infusion and the absence of flare in anti-RTX Abs weeks after OBZ infusion are both against this hypothesis.
Then we wondered if OBZ could enhance the formation of the cryoprecipitate leading to a flare of CV. To that end, we compared *in vitro* the density of the cryoprecipitate in sera collected in the patient before (Fig. 2A) and after (Fig. 2B) the post-OBZ flare. Sera were mixed with RTX, OBZ or tested without monoclonal antibody (negative control). We observed in sera mixed with RTX the formation of a denser cryoprecipitate than with OBZ or negative control after 7 days of incuba-

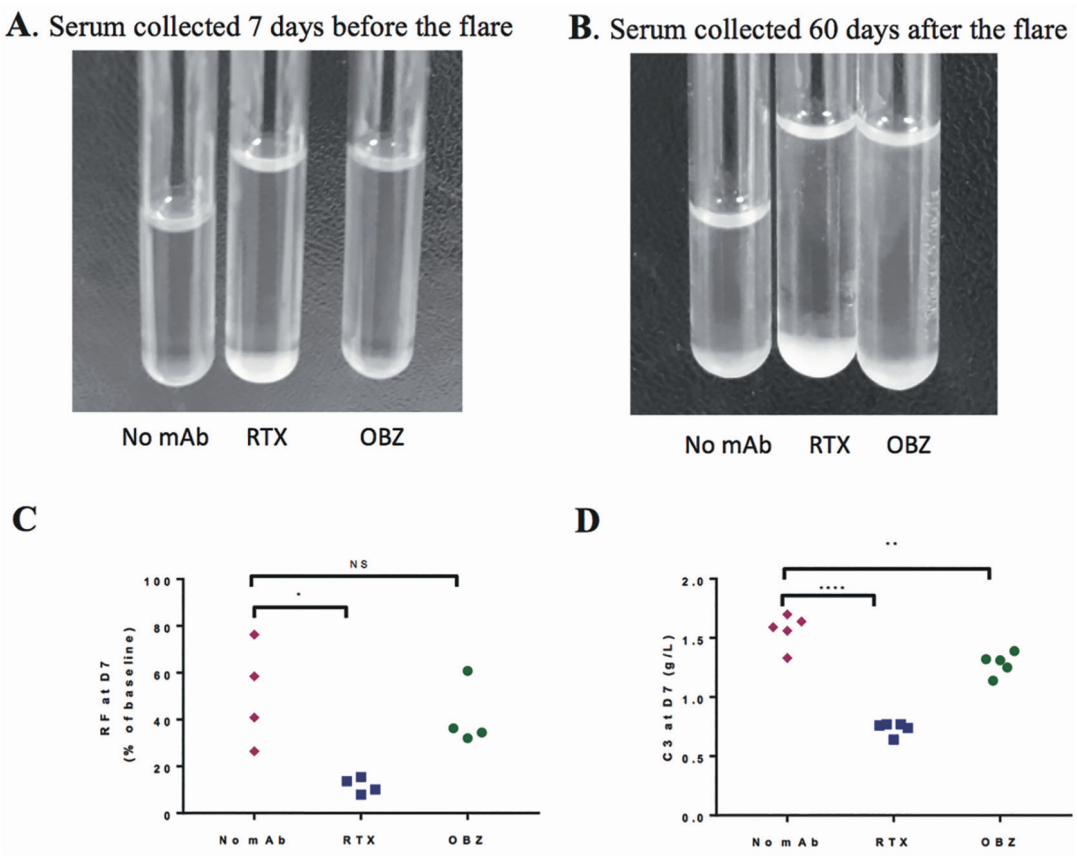

Fig. 2. Rituximab (RTX) induces the formation of a denser cryoprecipitate than obinutuzumab (OBZ) *in vitro* after a 7-day incubation in a patient with mixed cryoglobulinaemia.
Cryoprecipitate formation is shown 7 days post-incubation at 4°C in serum samples from the patient.
A was collected 7 days before the flare,
B was collected 60 days after the flare.
C and **D** show levels of rheumatoid factor (RF, as percentage of baseline RF) and complement C3 measured at day 7 (D7) in 4 sera from the patient collected before (n=3) or after (n=1) the flare. Sera were kept at 4°C without monoclonal antibody (no mAb) and with RTX or OBZ respectively and then RF and C3 were measured.
*p<0.05, **p<0.01, ***p<0.0001.
NS: not significant.

tion at 4°C, associated to significantly lower RF and C3 complement levels in the supernatant supporting higher cryo-precipitable immune complexes (IC) formation induced by RTX (Fig. 2C and 2D, respectively). The same experiment was performed with the serum of another patient with type II mixed cryoglobulinaemia but without anti-RTX Abs. In that case, no difference was detected in either three conditions. Based on these results, we could conclude that IC made of anti-RTX Abs and RTX increase cryoprecipitation in mixed cryoglobulinaemia.

Discussion

OBZ is an anti-B cell therapy approved in onco-haematology as an alternative to RTX. We herein describe the first case of vasculitis flare after OBZ infusion in a patient previously immunised against RTX. Subsequent treatment with anti-BAFF therapy lead to vasculitis remission, as previously described in SS (7, 8).

Infusion-related vasculitis flares in mixed cryoglobulinaemia have been described with RTX, typically between day 2 and 16 and always involving skin, as in our patient, but had not been observed with RTX or ofatumumab in the patient's clinical course (9). Thus, the absence of vasculitis flares under other anti-CD20 therapies does not seem to prevent from vasculitis flares under OBZ.

Furthermore, based on the complete B cell depletion and the lack of anti-RTX Abs after OBZ infusion, we conclude that there was no cross reactivity between anti RTX Abs and OBZ.

Then, we tried to determine if OBZ could enhance the formation of the cryoprecipitate leading to a flare of CV as it has been described with RTX (10). Based on the results of our experiments, we could conclude that IC made of anti-RTX Abs and RTX lead to higher cryoprecipitation. In clinical practice, this could provoke a flare of the vasculitis. Thus, presence of anti-RTX Abs needs to be carefully monitored in patients treated for CV especially in case of symptoms evoking anti-RTX immunisation such as previous reaction during infusion or incomplete B cell depletion.

However, these results did not explain the flare experienced by the patient after OBZ infusion. To explain this flare, we make the hypothesis that higher B cell death induced by OBZ could lead to the release of a high quantity of soluble CD20 that complexed with OBZ. This high amount of IC could react with cryoglobulin and provoke the flare of vasculitis as observed in this patient. We could not assess this hypothesis *in vitro,* but we think that based on this report, the use of OBZ needs to be carefully discussed in patients with mixed CV. Alternatively, a 4-week regimen with reduced weekly doses should be discussed. Association of anti-B cell with anti-BAFF therapy deserves further assessment.

References

1. COMBIER A, NOCTURNE G, HENRY J *et al.*: Immunization to rituximab is more frequent in systemic autoimmune diseases than in rheumatoid arthritis: ofatumumab as alternative therapy. *Rheumatology* 2020; 59: 1347-54.
2. COHEN AUBART F, HAROCHE J, AMOURA Z: More on Ofatumumab for TTP. *N Engl J Med* 2018; 378: 1364-5.
3. REDDY V, KLEIN C, ISENBERG DA *et al.*: Obinutuzumab induces superior B-cell cyto-

toxicity to rituximab in rheumatoid arthritis and systemic lupus erythematosus patient samples. *Rheumatology* (Oxford) 2017; 56: 1227-37.
4. CARTRON G, WATIER H: Obinutuzumab: what is there to learn from clinical trials? *Blood* 2017; 130: 581-9.
5. KLOMJIT N, FERVENZA FC, ZAND L: Successful treatment of patients with refractory PLA2R-associated membranous nephropathy with obinutuzumab: a report of 3 cases. *Am J Kidney Dis* 2020; 76: 883-8.
6. FELICETTI M, TREPPO E, POSARELLI C *et al*.: One year in review 2020: vasculitis. *Clin Exp Rheumatol* 2020; 38 (Suppl. 124): S3-14.
7. CHEVALIER K, BELKHIR R, SEROR R, MARIETTE X, NOCTURNE G: Efficacity of a sequential treatment by anti-CD 20 monoclonal antibody and belimumab in type II cryoglobulinaemia associated with primary Sjögren syndrome refractory to rituximab alone. *Ann Rheum Dis* 2020; 79: 1257-9.
8. GANDOLFO S, DE VITA S: Double anti-B cell and anti-BAFF targeting for the treatment of primary Sjögren's syndrome. *Clin Exp Rheumatol* 2019; 37 (Suppl. 118): S199-208.
9. DESBOIS AC, BIARD L, SÈNE D *et al*.: Rituximab-associated vasculitis flare: incidence, predictors, and outcome. *J Rheumatol* 2020; 47: 896-902.
10. SÈNE D, GHILLANI-DALBIN P, AMOURA Z, MUSSET L, CACOUB P: Rituximab may form a complex with iGmא mixed cryoglobulin and induce severe systemic reactions in patients with hepatitis C virus-induced vasculitis. *Arthritis Rheum* 2009; 60: 3848-55.