Sequential therapy with belimumab followed by rituximab in Sjögren's syndrome associated with B-cell lymphoproliferation and overexpression of BAFF: evidence for long-term efficacy

S. De Vita[1], L. Quartuccio[1], S. Salvin[1], L. Picco[1], C.A. Scott[2], M. Rupolo[3], M. Fabris[1,4]

[1]Clinic of Rheumatology, [2]Institute of Pathology, Department of Medical and Biological Sciences, University Hospital S. Maria della Misericordia, Udine, Italy; [3]Department of Oncology, Centro di Riferimento Oncologico, IRCCS-National Cancer Institute, Aviano, Italy; [4]Clinical Pathology, University Hospital S. Maria della Misericordia, Udine, Italy.

Abstract

Objective

The overexpression of B-cell activating factor (BAFF) in mucosa-associated lymphoid tissue (MALT) may decrease the efficacy of rituximab treatment in Sjögren's syndrome (SS). Anti-CD20 therapy was effective on marginal zone B cells, in the murine model for human CD20 expression only when preceded by anti-BAFF therapy. The possible efficacy of a sequential anti-BAFF/anti-CD20 therapy in SS was investigated.

Methods

We treated with belimumab, a monoclonal anti-BAFF antibody, and soon after with rituximab a patient with severe, refractory SS, parotid low-grade B-cell MALT lymphoma and cryoglobulinaemic vasculitis. Previous treatments with rituximab and with rituximab plus high dose glucocorticoids, as well as with cyclophosphamide, azathioprine, plasma exchange, hyperbaric therapy, VAC therapy, prostacyclin, mycophenolate mofetil and surgery, had previously failed. Treatment with belimumab was then given, but it also failed. A new course of rituximab (375 mg/m^2; four weekly infusions) was started 49 days after the last infusion of belimumab.

Results

This sequential belimumab-rituximab treatment was followed by a marked amelioration, with the complete and persistent regression of lymphoma and healing of a refractory skin ulcer. A full cycle of rituximab was then repeated 6 and 12 months later; no further treatment was given in the following 22 months up to now. Serum cryoglobulins and rheumatoid factor became persistently negative and serum BAFF and C4 persistently normal. No relevant side effects were noticed, except for a marked decrease in serum IgM. The follow-up after belimumab-rituximab sequential therapy is now three and a half years.

Conclusion

Therapy with belimumab followed by rituximab may be effective for SS-related B-cell lymphoproliferation. The efficacy and safety of the sequential or concomitant targeting of BAFF and CD20 deserves further evaluation in SS.

Key words

Sjögren's syndrome, lymphoma, cryoglobulinaemia, belimumab, rituximab

Salvatore De Vita, MD
Luca Quartuccio, MD, PhD
Sara Salvin, MD
Luca Picco, MD
Cathryn Anne Scott, MD, PhD
Maurizio Rupolo, MD
Martina Fabris, MD

Please address correspondence and reprint requests to:
Salvatore De Vita, MD,
Professor of Rheumatology,
Azienda Ospedaliero-Universitaria,
DSMB, Università di Udine,
P.z.le S. Maria della Misericordia 15,
33100 Udine, Italy.
E-mail: devita.salvatore@aoud.sanita.fvg.it

Received on December 21, 2013; accepted in revised form on January 9, 2014.

© Copyright CLINICAL AND EXPERIMENTAL RHEUMATOLOGY 2014.

Funding: Human Genome Science/Glaxo SmithKline provided belimumab and an unrestricted research grant for the BELISS study, which included the present case.

Competing interests: none declared.

Introduction

B-cell non-Hodgkin's lymphoma (NHL) complicates the course of about 5–10% of patients with Sjögren's syndrome (SS) and represents the principal cause of increased mortality in this disease (1, 2).

Effective treatments to modify the course of SS and its possible evolution into lymphoma are however lacking. Many different therapies, including heavy cytotoxic treatment for bone marrow transplantation, failed to improve chronic MALT inflammation in SS, which appears sustained by local, chronic antigenic triggering. Targeting the B cells with drugs lacking oncogenetic properties might be in any case beneficial, in particular for SS-related B-cell lymphoproliferation (3).

The hyperexpression of B-cell activating factor (BAFF) was shown in SS (4, 5): it may sustain the over-expansion of autoreactive B-cell clones in MALT, where it can also mediate the resistance to tissue B-cell depletion (6).

Disease heterogeneity, including a differential BAFF expression between patients, could underlie the conflicting results with rituximab treatment observed so far in SS (3, 6-9).

Belimumab, a monoclonal antibody against BAFF registered for the treatment of systemic lupus erythematosus (SLE) (10), was recently employed in SS with encouraging results (11, 12). Of note, in the murine model for human CD20 expression, marginal zone B cells were relatively resistant to anti-CD20 monotherapy, while they were very sensitive (>90% loss) to the simultaneous targeting of both CD20 and BAFF (6).

We herein report the long-term course of a patient with severe SS, low-grade parotid B-cell MALT lymphoma (MALT B-NHL), cryoglobulinaemic vasculitis, and BAFF hyperexpession, where previous monotherapy with rituximab and then with belimumab proved ineffective. Strikingly, when rituximab was administered again shortly after belimumab, a marked response was obtained.

Based on previous experimental data and the present novel clinical observations, treatment with belimumab shortly followed by rituximab is highlighted for investigation in SS, with particular regard to patients with heavy B-cell lymphoproliferation.

Materials and methods

A woman with SS, bilateral parotid low-grade MALT B-NHL and cryoglobulinaemic vasculitis, was studied.

In 1997, primary SS was diagnosed, at the age of 41, based on subjective and objective dry mouth and dry eye manifestations, positive anti-SSA and anti-SSB antibodies and positive minor salivary gland biopsy (1). Type II serum cryoglobulinaemia and positive rheumatoid factor (RF) were documented. Hepatitis B and C infection were absent.

In 2002, given the persistence of right parotid enlargement, a parotid biopsy was performed, showing a myoepithelial sialoadenitis with monoclonal B-cell expansion by molecular analyses.

In September 2003, bilateral parotid swelling persisted, while purpura and paraesthesia (axonal sensitive polyneuropathy by electromyography) had developed in the lower limbs, with a perimalleolar vasculitic skin ulcer of 2 x 3 cm of the right leg. A second right parotid biopsy showed this time a low-grade MALT B-NHL, stage IE by computed tomography (CT) and bone marrow biopsy. By sequence analyses, genes encoding for RF specificity (IGHV1-69*01/ IGHD6-13*01/ IGHJ4*02; KV3-20*01/KJ1*01) were used by the neoplastic clone (NCBI GenBank accession number: KF959633). The patient has been followed prospectively in the same Centre up to now.

Results

Rituximab monotherapy

In November 2003, four weekly infusions of rituximab 375 mg/m^2 were administered, with no clinical improvement of the parotid swelling, the skin ulcer, and polyneuropathy symptoms. Subsequently the patient failed cyclophosphamide and azathioprine. A third right parotid biopsy (July 2004) documented the persistence of the low grade MALT B-NHL. In February 2005, the patient underwent subtotal bilateral parotidectomy (bilateral low-grade MALT B-NHL) followed by a marked decrease in the size of the skin ulcer, the RF titre and serum cryoglobulins (14).

Eight courses of plasma exchange were then used, with complete healing of the ulcer. In September 2005, however, the skin ulcer and purpura recurred. Plasma exchange, using the previous schedule, was now ineffective. In February 2006, ultrasonography, CT and a fourth parotid biopsy confirmed the recurrence of the bilateral parotid low-grade MALT B-NHL, stage IE. BAFF serum levels were high and local expression of BAFF in the lymphomatous tissue was demonstrated (15).

Rituximab plus high dose glucocorticoids

In March 2006, eight consecutive rituximab weekly infusions of 375 mg/m^2 were given in association with high-dose glucocorticoids (GCs) (1 mg/kg/day of prednisone slowly reduced to 5 mg/day in five months) (15). No improvement was noticed regarding parotid enlargement (CT and ultrasonography), neuropathy symptoms and the skin ulcer. Serum BAFF, RF and cryoglobulins decreased but remained positive at high levels, and then increased with GC tapering (Table I) (15).

From August 2006 to May 2010, the patient was unsuccessfully treated with hyperbaric therapy, VAC therapy, intravenous prostacyclin, mycophenolate mofetil, and surgery (2 skin autografts).

Belimumab monotherapy

In June 2010, due to the persistence of parotid swelling, the skin ulcer and sensory neuropathy, the patient entered the BELISS study (11, 12) and started treatment with belimumab, 10 mg/kg at day 0, +14, +28 and then every month. Five mg/day of prednisone plus colchicine 1 mg/day were given concomitantly. A biopsy of lip minor salivary glands at study entry documented a low grade MALT B-NHL (Fig. 1), with monoclonal B-cell expansion by molecular studies.

The patient underwent a total of 5 infusions with belimumab, but the skin ulcer worsened; therefore belimumab was suspended. Furthermore, no decrease in parotid swelling was documented by ultrasonography. Serum cryoglobulins became negative, but the RF and BAFF decreased only slightly and C4 remained unchanged (Table I).

Rituximab therapy after belimumab

In November 2010, 49 days after the last infusion with belimumab, the patient underwent a third course of treatment with rituximab (375 mg/m^2, four weekly infusions), repeated with the same schedule 6 and 12 months later. No other treatment was given. The size of skin ulcer progressively decreased and completely healed 1 year later. The size of the parotid glands, assessed by ultrasonography every 6 months, gradually decreased and then persistently normalised from February 2012 (Table I). Mild neuropathy symptoms persisted, with no worsening by repeated electromyography.

Small salivary gland biopsy was repeated before the first retreatment with rituximab (July 2011) and 16 months following suspension of rituximab treatment (May 2013) (Fig. 1). In July

Table I. Summary of the clinical, laboratory and histologic features of the patient's history.

Therapy	2003 Nov RTX pre/post	2006 Mar RTX + high dose GCs pre/post	2010 Jun BEL pre/post	2010 Nov RTX pre/post	2011 July RTX pre/post	2012, Jan RTX pre/post	2013 Oct Last follow-up
Clinic							
Parotid swelling (ultrasound width*)	+/+ (25 mm/24 mm)	+/+	+/+	+/↓ (24 mm/18 mm)	↓/↓↓ (18 mm/13 mm)	↓↓/- (13 mm/7.5 mm)	- (7.5 mm)
Skin ulcer	+/+	+/+	+/+	+/↓	↓↓/↓	-/-	-/-
Peripheral neuropathy	+/+	+/+	+/+	+/+	+/+	+/+	+/+
Histology							
Parotid biopsy	Low grade MALT B-NHL						
Lip biopsy			Low grade MALT B-NHL		Sialadenitis focus score 1,648	Sialadenitis focus score 0,759	
Laboratory							
Cryoglobulins	+/+	+/+	+/-	+/-	-/-	-/-	-
RF (IU/l)	9190/23500	13400/866	638/413	413/109	95/77	72/-	-
Serum monoclonal component	IgM k/IgM k	IgM k/IgM k	IgM k/IgM k	IgM k/IgM k	IgM k/-	-/-	-
C4 (mg/dl)	7/7	5/5	0/1	1/6	8/15	19/25	51
BAFF (pg/ml)	2038/3866	3961/7726	13512/13064	14271/19580	2580/4194	2058/918	759
IgG (mg/dl)	3249/3412	2448/941	1529/1128	1128/895	851/838	862/787	811
IgA (mg/dl)	346/409	374/240	254/213	213/192	188/192	197/172	158
IgM (mg/dl)	1351/1107	466/156	295/157	157/52	51/40	35/5	2
Blood B-cells (CD19+)	7%/neg	5,5%/neg	33%/11%	11%/neg	neg/neg	neg/neg	neg/neg
ANA titre	1:1280	1:1280	1:1280	1:1280	1:1280	1:640	1:640
Anti-SSA/SSB Abs	+/+	+/+	+/+	+/+	+/+	+/+	+/+

RTX: Rituximab; GCs: Glucocorticoids; BEL: Belimumab; RF: Rheumatoid Factor; ↓: <25% decrease in skin ulcer diameter or parotid gland enlargement from baseline; ↓↓: 25%–75% decrease in skin ulcer diameter or parotid gland enlargement from baseline.
*The width of the right parotid gland was measured in the transversal plane using a 6-18 MHz linear scanner. Normal values: BAFF <1000 pg/mL.

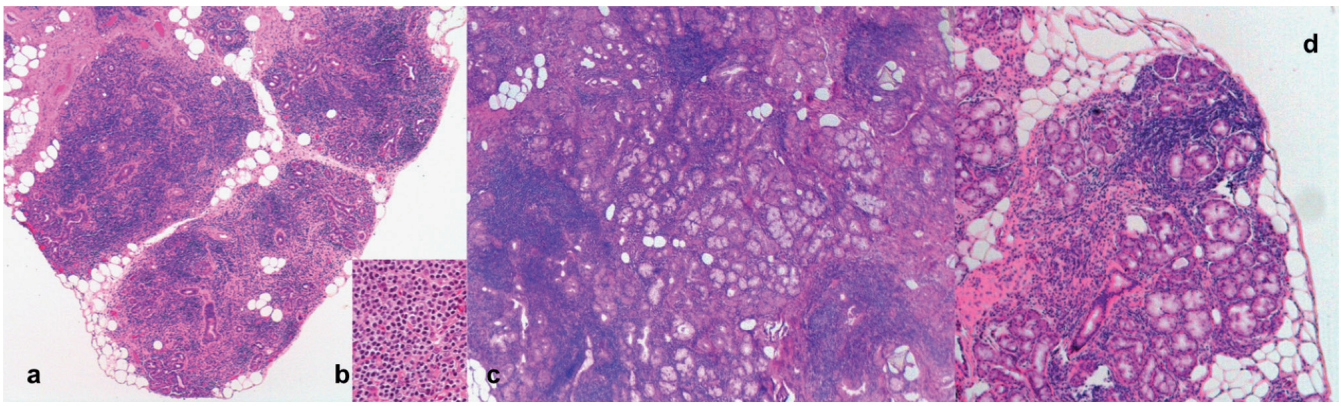

Fig. 1.
a) Small salivary gland biopsy in June 2010: a diagnosis of Non Hodgkin B cell lymphoma was rendered of MALT type. At low power examination (a: 4 x original magnification) a diffuse infiltration by small lymphocytes is seen associated with extensive acinar depletion; focus score could not be calculated because of the confluence of the lymphoid population that showed at high power magnification (inset **b**: 40x original magnification) a proliferation of small centrocytic-like lymphocytes with clear cytoplasm. Epi-myoepithelial islands (not shown) were also present.
c) Small salivary gland biopsy in July 2011: a diagnosis of chronic sialoadenitis and epi-myoepithelial islands was rendered. At low power examination (4 x original magnification) non- confluent foci of small lymphocytes are evident within which some lymphoid follicles are appreciated; acinar depletion is present, but much less severe than in the previous biopsy; focus score corresponded to 1,648.
d) Small salivary gland biopsy in June 2013: a diagnosis of chronic sialoadenitis was rendered.
At intermediate power magnification (20 x original magnification) a small lymphocytic focus is present. Little damage is seen to acini and striated ducts and the focus score corresponded to 0.759. No epi-myoepithelial islands are evident.

2011 the biopsy showed no morphological evidence of MALT B-NHL, the focus score was 1,648, with no light chain restriction by immunohistochemistry and no tissue monoclonal B-cell expansion by molecular studies (Fig. 1). In May 2013 the focus score further decreased to 0.759 with no epymyoepithelial islands (Fig. 1).
Table I and Figure 2 show the variations in serum cryoglobulins (persistently negative), serum RF (slow decrease and persistent negativity from March 2013), total gammaglobulins (within normal range, with decrease below normal of IgM from December 2010), peripheral blood B-cells (persistently depleted), anti-nuclear antibodies and anti-SSA/SSB (persistently unchanged), and serum BAFF markedly increased only after the initial rituximab cycle, while later it decreased and finally normalised (Table I; Fig. 2). From March 2012 to date, the patient is no longer undergoing treatment for SS.

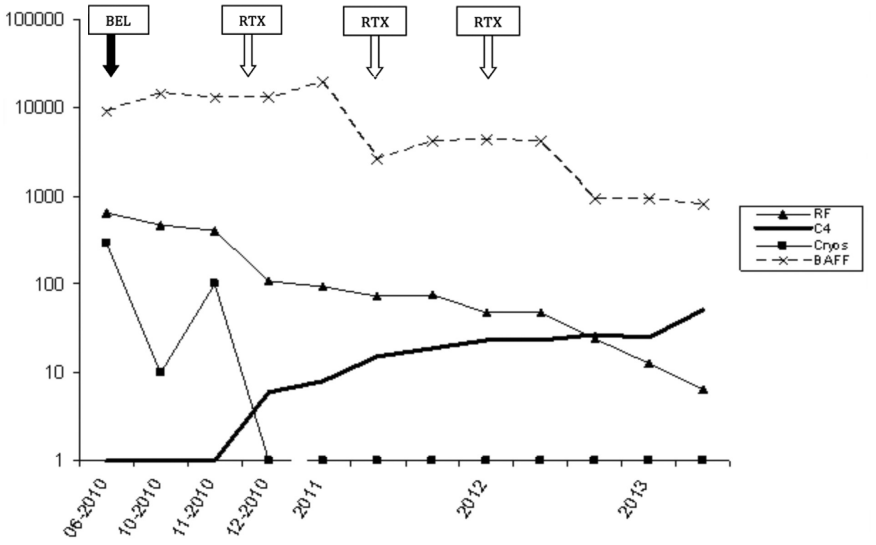

Fig. 2. The course of serum cryoglobulins (mg/dl), rheumatoid factor (IU/ml), C4 (mg/dl), and serum BAFF (pg/ml) after sequential belimumab/rituximab therapy.

Discussion

In the present paper we highlight the possible usefulness of a sequential therapy with belimumab shortly followed by rituximab in SS-related B-cell lymphoproliferation.
B-cell overexpansion characterises SS, and B-cell depletion therapy with rituximab is then rationale. Very early experience by our Group with this drug, however, failed to show a convincing effect in some severe cases, with B cells being still heavily represented in MALT after treatment (3, 7). The results with rituximab in SS remain conflicting at present (3, 7-9), consistent with a biologic heterogeneity of the disease between patients, possibly including a differential expression of local growth factors mediating drug resistance.
Of note, the local expression of BAFF in MALT may limit the effect of rituximab, and targeting BAFF might facilitate the action of this drug, as demonstrated in the murine model for human CD20 expression, where anti-CD20 therapy proved very effective on marginal zone B cells only when associated with anti-BAFF therapy (6). BAFF is increased in the serum of a fraction of patients with SS, and is believed to play an important pathogenetic role. Furthermore, higher serum BAFF levels were detected in the course of heavier

lymphoproliferation and more active SS (5). Treatment with belimumab, an anti-BAFF monoclonal antibody registered for SLE (10), was recently used in monotherapy in the BELISS trial in SS (11, 12) that showed a beneficial clinical effect, in particular, in SS patients with parotid swelling (12). A biologic effect on serum biomarkers SS-related B-cell lymphoproliferation was also seen (11, 12). Drug safety appeared good in SS, similarly to SLE (10-12).

One of the patients of the BELISS trial is the patient herein reported. BAFF was hyperexpressed both in the serum and the tissue of our patient (15), but belimumab therapy failed in this severe case. The evidence that rituximab had failed when given before belimumab, while it was effective after belimumab, is fundamental to support the possible usefulness of the treatment sequence with rituximab preceded by belimumab, herein highlighted.

After the failure of the first rituximab course, and given the severity of disease, a second attempt was carried out associating a more prolonged rituximab therapy (16) with high dose GCs that provide B-cell inhibition and may decrease BAFF (13). BAFF production, however, could not be abrogated, and the rituximab plus high-dose GCs combination was ineffective (15). Bilateral subtotal parotidectomy proved useful also for the course of vasculitis (14), but parotid lymphoma later recurred and vasculitis worsened. Finally, when rituximab was administered for the third time, but shortly after the failure of belimumab, a persistent clinical and biologic efficacy was noticed with lack of clinically relevant side effects. Of note, after an initial increase, serum BAFF decreased and finally became normal from 2013.

The present patient is reported, after a long-term follow-up, to highlight the possible benefits of a belimumab-rituximab sequential treatment for subsets of patients with SS. Our findings also underscore that the optimal drug sequence may vary in different autoimmune diseases with a different pathophysiology. The use of belimumab after rituximab, rather than before, has been considered in SLE. Although the role of repeated courses of rituximab in achieving further disease improvement and stabilisation is likely in the present case, previous treatment with belimumab appeared crucial.

With the disappearance of risk factors of lymphoma in SS (cryoglobulins and low C4), belimumab-rituximab/anti-BAFF-anti-CD20 sequential therapy could effectively target the RF-positive lymphoma-related lymphoproliferation (1, 2, 13). On the other hand, since lymphoproliferation is in any case dependent on the underlying autoimmune disorder, the possible benefits of BAFF down regulation before (or concomitantly with) (6) anti-CD20 therapy could be explored also for the whole group of SS patients. Safety issues, in particular the short- and long-term effects on humoral immunity, deserve a very careful evaluation.

Acknowledgments

We acknowledge the contribution of Human Genome Science/Glaxo Smith-Kline in providing belimumab and an unrestricted research grant for the BELISS study, which included the present case.

References

1. TZIOUFAS AG, VOULGARELIS M: Update on Sjögren's syndrome autoimmune epithelitis: from classification to increased neoplasias. *Best Pract Res Clin Rheumatol* 2007: 21: 989-1010.
2. QUARTUCCIO L, ISOLA M, BALDINI C *et al*.: Biomarkers of lymphoma in Sjögren's syndrome and evaluation of the lymphoma risk in prelymphomatous conditions: results of a multicenter study. *J Autoimmunity* 2013 Nov 11 [Epub ahead of print].
3. QUARTUCCIO L, FABRIS M, SALVIN S, MASET M, DE MARCHI G, DE VITA S: Controversies on rituximab therapy in Sjögren's syndrome-associated lymphoproliferation. *Int J Rheumatol* 2009; 2009: 424935.
4. LAVIE F, MICELI-RICHARD C, QUILLARD J, ROUX S, LECLERC P, MARIETTE X: Expression of BAFF (BlyS) in T cells infiltrating labial salivary glands from patients with Sjögren's syndrome. *J Pathol* 2004; 202: 496-502.
5. QUARTUCCIO L, SALVIN S, FABRIS M *et al*.: BlyS upregulation in Sjögren's syndrome associated with lymphoproliferative disorders, higher ESSDAI score and B-cell clonal expansion in the salivary glands. *Rheumatology* (Oxford) 2013; 52: 276-81.
6. GONG Q, OU Q, YE S *et al*.: Importance of cellular microenvironment and circulatory dynamics in B cell immunotherapy. *J Immunol* 2005; 174: 817-26.
7. DE VITA S, DE MARCHI G, SACCO S, ZAJA F, SCOTT CA, FERRACCIOLI G: Treatment of B-cell disorders of MALT in Sjögren's syndrome with anti-CD20 monoclonal antibody. The VIIIth international symposium on Sjögren's syndrome, Kanazawa, Japan, May 2002; P8-2, page 51. [Abstract].
8. DASS S, BOWMAN SJ, VITAL EM *et al*.: Reduction of fatigue in Sjögren syndrome with rituximab: results of a randomized, double-blind, placebo-controlled pilot study. *Ann Rheum Dis* 2008; 67: 1541-4.
9. MEKINIAN A, RAVAUD P, LARROCHE C *et al*.; CLUB RHUMATISMES ET INFLAMMATION: Rituximab in central nervous system manifestations of patients with primary Sjögren's syndrome: results from the AIR registry. *Clin Exp Rheumatol* 2012; 30: 208-12.
10. MOSCA M, VAN VOLLENHOVEN R: New drugs in systemic lupus erythematosus: when to start and when to stop. *Clin Exp Rheumatol* 2013; 31 (Suppl. 78): S82-5.
11. MARIETTE X, QUARTUCCIO L, SEROR S *et al*.: Results of the Beliss Study, the First Open Phase 2 Study of Belimumab in Primary Sjögren's Syndrome. *Ann Rheum Dis* 2013 Dec 17 [Epub ahead of print].
12. DE VITA S, SEROR R, QUARTUCCIO L *et al*.: Efficacy of belimumab on non-malignant parotid swelling and systemic manifestations of Sjögren's syndrome results of the BELISS study. *Arthritis Rheum* 2012; 64: S926.
13. DE RE V, DE VITA S, SANSONNO D *et al*.: Type II mixed cryoglobulinaemia as an oligo rather than a mono B-cell disorder: evidence from GeneScan and MALDI-TOF analyses. *Rheumatology* (Oxford) 2006; 45: 685-93.
14. DE VITA S, QUARTUCCIO L, SALVIN S, CORAZZA L, ZABOTTI A, FABRIS M: Cryoglobulinaemia related to Sjögren's syndrome or HCV infection: differences based on the pattern of bone marrow involvement, lymphoma evolution and aboratori tests after parotidectomy. *Rheumatology* (Oxford) 2012; 51: 627-33.
15. QUARTUCCIO L, FABRIS M, MORETTI M *et al*.: Resistance to rituximab therapy and local BAFF overexpression in Sjögren's syndrome-related myoepithelial sialadenitis and low-grade parotid b-cell lymphoma. *Open Rheumatol J* 2008; 2: 38-43.
16. ISHIYAMA K, TAKAMI A, OKUMURA H *et al*.: Complete and durable remission of refractory mantle cell lymphoma with repeated rituximab monotherapy. *Int J Hematol* 2005; 81: 319-22.